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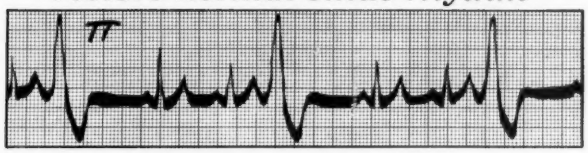

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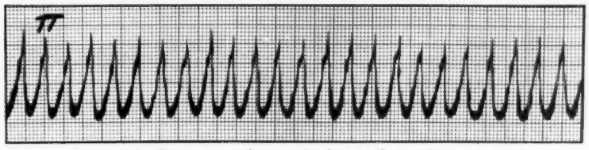

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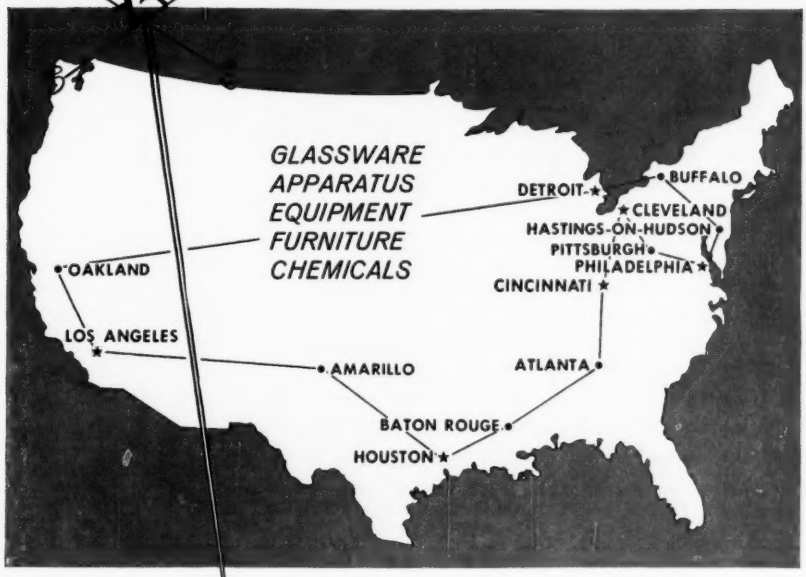

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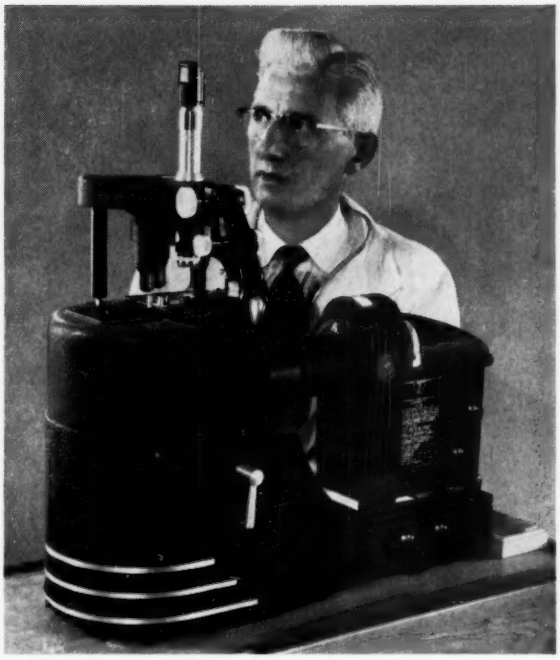

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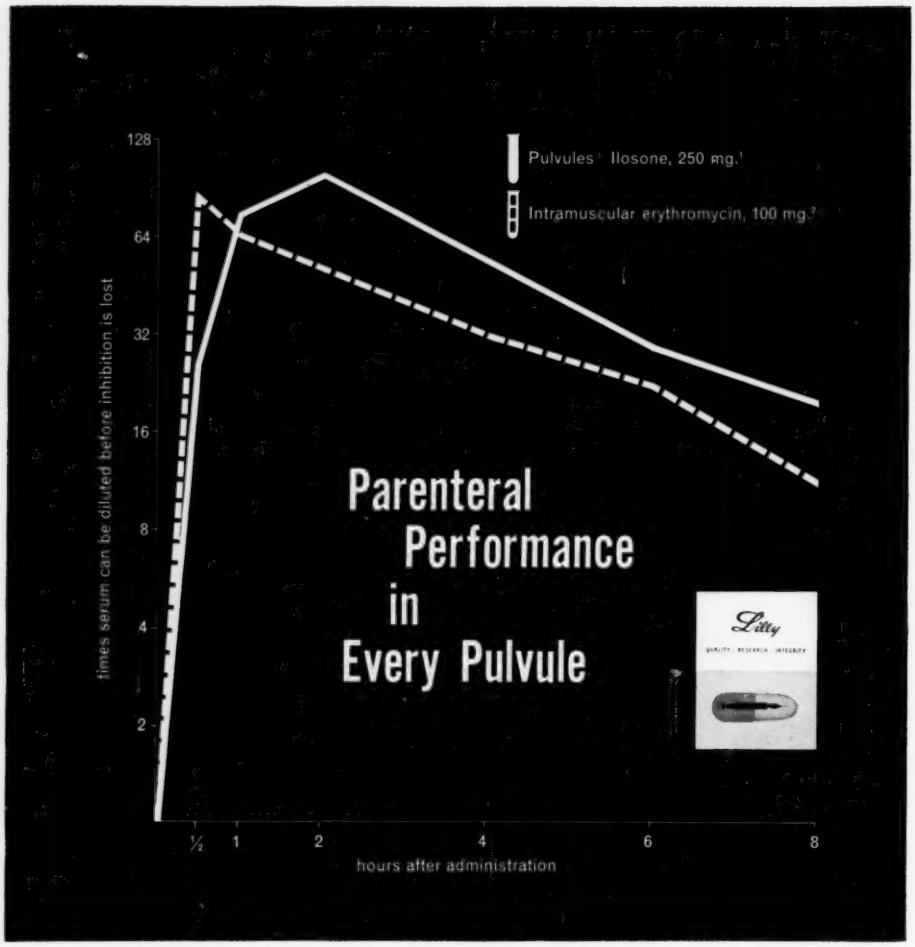

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METABOLISM OF MUCOPOLYSACCHARIDES IN CONNECTIVE TISSUE. I. STUDIES OF ENZYMES INVOLVED IN GLUCURONIDE METABOLISM *

By ALFRED JAY BOLLET,† JESSE F. GOODWIN AND AUDREY K. BROWN

(From Wayne State University College of Medicine, and Detroit Receiving Hospital, Detroit, Mich.)

(Submitted for publication October 6, 1958; accepted October 23, 1958)

Details of the intermediary metabolism of mucopolysaccharides have been studied in microorganisms and some animal tissues (1). We have undertaken an exploration of enzymatic steps in the synthesis of mucopolysaccharides in extracts of the connective tissue which enter polyvinyl sponges inserted under the skin of guinea pigs and extracts of human synovial tissue obtained surgically.

Uridine diphosphoglucuronic acid (UDPGA) is presumed to be the donor of glucuronic acid in the synthesis of acid mucopolysaccharides (1). This report concerns primarily the enzymatic steps involved in the synthesis of UDPGA from glucose, and other enzymes involved in the metabolism of glucuronides, including glucuronosyl transferase and beta-glucuronidase. Schematic outline of the actions of the enzymes studied is given in Table I.

METHODS

Polyvinyl (Ivalon®) sponges were inserted under the dorsal skin of male guinea pigs, weighing 500 ± 100 Gm., anesthetized with pentobarbital (20 mg. per Kg.). The animals were fed standard Purina guinea pig chow. After two to four weeks, they were killed by a blow on the head, and the sponges and liver were removed and homogenized separately. Synovial tissue was obtained from human beings at the time of arthrotomy for orthopedic surgery or on removal of ganglia. All steps were carried out at 0 to 4° C. Activities are expressed per milligram of protein, except where indicated.

1. Hexokinase. a) Preparation. The 0 to 50 per cent ammonium sulfate fraction from particle free supernatant was prepared following the procedure described by Crane and Sols (2) for liver hexokinase.

b) Assay. Aliquots of the enzyme preparations were assayed by two methods. After incubation under the conditions specified by Crane and Sols (2), the change in acid-labile phosphorus was determined following hydrolysis in 5 N H_2SO_4 for five minutes at 100°. Phosphorus determinations were done by the method of Goodwin (3). As a second method of assay, the entire incubation mixture was added to a solution containing 0.6 mM of glycylglycine buffer, pH 7.4, 0.2 μM of triphosphopyridine nucleotide (TPN), 0.01 mM of $MgCl_2$ and 0.0015 unit of glucose-6-phosphate dehydrogenase (Sigma, Type III) in a total volume of 1.9 ml. The subsequent reduction of TPN to TPNH was read at 340 mμ in a Beckman DU spectrophotometer. In both procedures, controls consisted of the same incubation mixture heat-inactivated at zero time, and incubation carried out with glucose omitted.

2. Phosphoglucomutase. a) Preparation. The initial steps in the procedure described by Najjar (4) were followed; extracts were made in four volumes of distilled water, heated to 65° C., and a 0 to 65 per cent ammonium sulfate fraction obtained. Assays were done within 30 hours of the start of the preparation.

b) Assay. The method of Najjar (4) was used; glucose-1-phosphate conversion to glucose-6-phosphate was determined by the fall in acid-labile phosphorus by the method of Goodwin (3). Controls consisted of the same systems heat-inactivated at zero time.

3. Uridine diphosphoglucose (UDPG) pyrophosphorylase and dehydrogenase. a) Preparation. Forty to 55 per cent ammonium sulfate fraction was obtained from water extracts of the tissue, following the initial two steps in the procedure of Strominger, Maxwell, Axelrod and Kalckar (5) for the purification of UDPG dehydrogenase. This fraction was suspended in 0.02 M acetate buffer, pH 5.4, and dialyzed against that buffer for five hours.

b) Assay of UDPG pyrophosphorylase. One-tenth μM of uridine triphosphate, 20 μM of glucose-1-phosphate, 5 μM of diphosphopyridine nucleotide (DPN), 250 μM glycine buffer, pH 8.7, and 5 units of purified UDPG dehydrogenase (Sigma), in a total volume of 0.8 ml., were placed in microcuvettes; 0.1 ml. of the enzyme preparation to be tested was added. The formation of reduced diphosphopyridine nucleotide (DPNH) was determined spectrophotometrically at 340 mμ in a Beckman model DU spectrophotometer at room temperature. Controls included similar systems with uridine triphosphate omitted. In this and other controls run with glucose-1-phosphate omitted or with uridine diphosphate

* Supported by grants from the Michigan Chapter of The Arthritis and Rheumatism Foundation; the Receiving Hospital Research Corporation; and the National Institutes of Health, United States Public Health Service (Grant RG-5105).

† John and Mary R. Markle Scholar in Medical Science.

TABLE I

Outline of enzymatic reactions studied in connective tissue *

Enzyme	Reaction
Hexokinase	Glucose + ATP → Glucose-6-PO_4 + ADP
Phosphoglucomutase	Glucose-6-PO_4 ⇌ Glucose-1-PO_4
UDPG pyrophosphorylase	Glucose-1-PO_4 + UTP ⇌ UDPG + PP
UDPG dehydrogenase	UDPG + 2 DPN → UDPGA + 2 DPNH + 2 H^+
Glucuronosyl transferase	R-OH + UDPGA → R-GA + UDP
Beta-glucuronidase	R-GA + HOH → R-OH + GA
Cytochrome c reductase	DPNH + 2 Cytochrome c (Fe^{+++}) → DPN^+ + 2 Cytochrome c (Fe^{++}) + H^+

* Abbreviations used: ATP = adenosine triphosphate; ADP = adenosine diphosphate; UTP = uridine triphosphate; UDPG = uridine diphosphoglucose; UDPGA = uridine diphosphoglucuronic acid; PP = pyrophosphate; DPN = diphosphopyridine nucleotide; DPNH = reduced diphosphopyridine nucleotide; GA = glucuronic acid.

substituted for the uridine triphosphate, very little reduction of DPN occurred. Activity was expressed as micromoles of DPN reduced per minute during the second to sixth minutes of incubation, correcting for any DPN reduction in controls run simultaneously.

c) Assay of UDPG dehydrogenase. The formation of DPNH was determined spectrophotometrically, following the procedure of Strominger, Maxwell, Axelrod and Kalckar (5). Controls consisted of the same incubation mixture with UDPG omitted. Activity was expressed in the same manner as was used for UDPG pyrophosphorylase.

5. Glucuronosyl transferase. a) Preparation. Homogenates were made in 0.25 M sucrose buffered to pH 7.6. For some experiments, the sediment obtained by ultracentrifugation between 37,000 and 80,000 × G was used, suspended in 0.25 M sucrose.

b) Assay using o-aminophenol as acceptor. The sediment obtained by ultracentrifugation was incubated with 0.2 μM of UDPGA (Sigma), 0.1 mM of $MgCl_2$, 0.55 μM of recrystallized *o*-aminophenol and 0.035 mM tris(hydroxymethyl) aminomethane (tris) buffer, pH 7.6, in a total volume of 1.0 ml. After incubation at 37° C. for 45 minutes, *o*-aminophenol glucosiduronic acid formation was determined by the method of Levvy and Storey (6).

c) Assay using 4-methyl umbelliferone as acceptor.[1] Homogenate in sucrose was incubated for 15 minutes at 37° C. with 0.2 μM of UDPGA, 0.1 Gm. of $MgCl_2$, 0.35 mM of tris buffer and 0.2 μM of 4-methyl umbelliferone in a total volume of 2.2 ml. The incubation mixture was then shaken with 10 ml. of chloroform twice to denature protein and extract unconjugated umbelliferone. Aliquots of 0.5 ml. of the incubation mixture were then incubated for one hour at 37° C. with 0.2 mM of tris buffer, pH 7.0, and 50 units of bacterial beta-glucuronidase (Sigma) in a total volume of 2.0 ml. Five ml. of glycine buffer, 0.5 M, pH 10.35, was then added and fluorescence determined and compared to the fluorescence of similar aliquots carried through the same procedure without beta-glucuronidase. The increase in fluorescence following incubation with beta-glucuronidase was considered to indicate the amount of umbelliferone which was conjugated with glucuronic acid in the initial incubation.

6. Beta-glucuronidase. a) Preparation. Assays were done using homogenate suspended in 0.25 M sucrose buffered to pH 7.6.

b) Assay. The procedure of Fishman and Bernfeld (7) was followed. Results are expressed as micrograms

[1] The assay procedure used was based on suggestions of Dr. Gordon Tompkins of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md. This method was found to give results proportional to the amount of protein in the incubation mixture only when small amounts of enzyme activity were present. Liver homogenates were diluted so that only one mg. of protein, or less, was used in the assay.

TABLE II

Clinical data on human synovial tissue samples studied

Tissue sample	Source	Diagnosis	Sex	Age
A	Hip	Osteoarthritis secondary to aseptic necrosis of femoral head	M	38
B	Knee	Charcot joint	F	39
C	Knee	Torn meniscus	M	24
D	Tendon sheath	Ganglion	F	33
E	Tendon sheath	Ganglion (pooled tissue from 2 patients)		
F	Knee	Fracture of patella	F	57
G	Hip	Rheumatoid arthritis	M	32
H	Knee	Rheumatoid arthritis	M	50

TABLE III

UDPG pyrophosphorylase and UDPG dehydrogenase activity of 40 to 55 per cent ammonium sulfate fraction obtained from extracts of guinea pig tissue and human synovial tissue *

Tissue sample	UDPG pyrophosphorylase			UDPG dehydrogenase		
	Mean	S.E.M.†	No. of observations	Mean	S.E.M.†	No. of observations
A. Guinea pig tissues						
Sponge tissue	0.5	±0.18	10	1.5	±0.18	13
Liver	3.0	±0.41	8	13.9	±1.30	10
B. Human synovial tissues						
A	0.2			0.3		
B	0.02			0.5		
H	0.8			0.7		

* Figures given are micromoles ($\times 10^{-3}$) of diphosphopyridine nucleotide reduced per minute per mg. of protein.
† S.E.M. = standard error of the mean.

of phenolphthalein released from phenolphthalein glucuronide after one hour at 37° C.

7. Cytochrome c reductase. a) Preparation. Assays were done on homogenate suspended in 0.25 M sucrose buffered to pH 7.6.

b) Assay. The method described by Mahler (8) was used; 2,6-dichlorphenol indophenol was used as the electron acceptor. The incubation mixture consisted of 1.3 ml. of 0.05 M phosphate buffer, pH 7.4, containing 0.001 M KCN, 0.18 μM of dye, 0.6 μM of DPNH, and homogenate in a total volume of 2.6 ml. Dye reduction was determined in a Beckman DU spectrophotometer at 600 mμ at room temperature. Results are reported as micromoles of dye reduced between the thirtieth and one hundred twentieth seconds of incubation.

8. Protein determination. Protein in the samples assayed was estimated by the determination of ultraviolet absorption at 280 and 260 mμ, as described by Layne (9).

RESULTS

The figures given for guinea pig tissues are mean values, while the figures given for human synovial tissues are individual determinations; clinical data for these cases are given in Table II.

Hexokinase. The fraction prepared from sponge granulomas was found to have hexokinase activity, demonstrated by the transfer of acid-labile phosphorus of adenosine triphosphate (ATP) to acid-stable phosphorus of glucose-6-phosphate. In the assay procedure used, 80 μM of ATP was converted per gram of sponge tissue extracted.

Phosphoglucomutase. Sponge tissue converted an average of 64×10^{-2} μM of glucose-1-phosphate to glucose-6-phosphate per gram of tissue extracted, under the conditions used.

UDPG pyrophosphorylase and UDPG dehydrogenase. The active fractions obtained from sponge tissues were found to have 17 per cent of the UDPG pyrophosphorylase, and 11 per cent of the UDPG dehydrogenase activity of similar fractions isolated from the livers of the same animals (Table III). Following incubation, the mixtures used in the assay of the UDPG pyrophosphorylase and dehydrogenase from sponge and liver tissue were adjusted to pH 3.1, and adsorbed with charcoal (Norit A). The charcoal was eluted with ethanol containing 1:1,000 ammonium hydroxide, and a carbazole procedure was run on the eluates according to the method of Dische (10) to demonstrate the formation of glucuronic acid. A pink color with an absorption spectrum corresponding to that of glucuronolactone was found in each instance.

Activity of these enzymes was also demonstrated in human synovial tissue (Table III).

TABLE IV

Glucuronosyl transferase activity of guinea pig tissue and human synovial tissue, using 4-methyl umbelliferone as acceptor

Tissue sample	Amount conjugated ($\mu M \times 10^{-4}$ per mg. protein)		
	Mean	S.E.M.	No. of observations
A. Guinea pig tissues			
Sponge tissue	2.3	±0.54	8
Liver	110.0	±7.7	8
B. Human synovial tissues			
C	0.5		
D	1.6		

TABLE V

Beta-glucuronidase activity of human synovial tissue

Tissue sample	μg. of phenolphthalein released per mg. of protein
A	10.2
B	12.1
C	11.0
D	9.0
F	12.5
G	15.8
H	14.0

Glucuronosyl transferase. Conjugation of *o*-aminophenol to *o*-aminophenol glucosiduronic acid took place when the ultracentrifugational sediment obtained from sponge tissue extracts was used as the source of glucuronosyl transferase; 9.6×10^{-3} μM of substrate was conjugated per gram of sponge tissue. This activity was 7 per cent of the activity of the same fraction isolated from the liver. Using crude homogenates of sponge extracts and 4-methyl umbelliferone as the acceptor, glucuronosyl transferase activity was again demonstrable. With this system the sponge tissue was found to have 2.1 per cent of the activity of liver homogenates and this enzyme was also found in homogenates of human synovial tissue (Table IV).

Beta-glucuronidase. Sponge tissue homogenates were found to release an average 18.6 μg. (standard error ± 2.6) of phenolphthalein per mg. of protein; homogenates of the liver of the same 10 animals released an average of 11.9 ± 1.1 μg. of phenolphthalein per mg. of protein. Synovial tissue homogenates also had beta-glucuronidase activity (Table V).

DISCUSSION

The intermediary metabolism of the mucopolysaccharides of connective tissue has been studied in a variety of microorganisms and some vertebrate tissues, but has not been studied previously in mammalian connective tissue. Granulation tissue proliferation can be induced in experimental animals by the insertion of polyvinyl sponges (11). Fibroblasts, small vessels and a few giant cells appear in these sponges without inflammatory cell infiltration; the mucopolysaccharide concentration in the sponges reaches a peak at two weeks (12). This tissue provides a means of investigation of mucopolysaccharide metabolism in mammalian connective tissue.

The finding in connective tissue of the enzymes necessary to synthesize UDPGA from glucose supports the likelihood that UDPGA is an intermediary in the synthesis of the glucuronic acid moiety of mucopolysaccharides. This possibility has been suggested by the observation of Glaser and Brown (13) that labeled UDPGA is incorporated into hyaluronic acid by the Rous chicken sarcoma.

The relatively high concentration of beta-glucuronidase in the sponge tissue is of interest in view of observation by Linker, Meyer and Weissmann (14) that this enzyme hydrolyzes glucuronidic bonds in oligosaccharides derived from hyaluronic acid. Since hepatic glucuronosyl transferase is considered the enzyme involved in the synthesis of glucuronides of a variety of phenolic, alcoholic and steroid compounds, with UDPGA serving as the donor of glucuronic acid (15), the presence of this enzyme in connective tissue leads to speculation concerning its biologic role in that tissue. Fishman and Green (16) have suggested that béta-glucuronidase can serve to transfer glucuronic acid from preformed glucuronides to a variety of acceptors. If this mechanism is operative *in vivo*, the glucuronosyl transferase might form glucuronides which serve as substrate for this reaction. These considerations, however, only serve to underscore the lack of information concerning the enzyme systems involved in the synthesis of oligosaccharides and the need for further investigation.

Cytochrome c reductase was measured as an index of general metabolic activity of the tissues

TABLE VI

Cytochrome c reductase activity of guinea pig tissue and human synovial tissue

Tissue sample	μM dye reduced per mg. protein		
	Mean	S.E.M.	No. of observations
A. Guinea pig tissues			
Sponge tissue	1.7	±0.55	4
Liver	7.0	±0.67	5
B. Human synovial tissues			
B	0.7		
G	1.1		
H	1.1		

studied, to serve as a basis for comparison of the activity of enzymes involved in other metabolic functions. Such an index may be particularly useful in studies of connective tissue enzymes, where the relatively large amount of extracellular protein dilutes intracellular enzymes in tissue homogenates (Table VI).

Synovial tissue slices have been shown to be capable of the synthesis of mucopolysaccharide from glucose (17). The present study demonstrates activity of individual enzymes involved in mucopolysaccharide metabolism in extracts of human synovial tissue. The presence of measurable activity of these enzymes permits investigation of connective tissue metabolism in various diseases and exploration of mechanisms of action of therapeutic agents.

SUMMARY

Activity of enzymes involved in mucopolysaccharide metabolism can be demonstrated in extracts of connective tissue grown in polyvinyl sponges inserted under the dorsal skin of guinea pigs and in extracts of human synovial tissue. Enzymes involved in the synthesis of uridine diphosphoglucuronic acid (UDPGA) from glucose were studied, as were glucuronosyl transferase, beta-glucuronidase and cytochrome c reductase. The techniques used permit comparison of mucopolysaccharide metabolism in synovial tissue in various clinical states.

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THE INFLATIONARY FORCE PRODUCED BY PULMONARY VASCULAR DISTENTION IN EXCISED LUNGS. THE POSSIBLE RELATION OF THIS FORCE TO THAT NEEDED TO INFLATE THE LUNGS AT BIRTH

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Among the forces that may contribute to the first inflation of the lungs after birth, those developed through changes in the pulmonary circulatory system have been assigned primary importance by Jäykkä (1), Bonham Carter (2) and Adams, Karlberg and Lind (3). Possibly the earliest basis for this view was provided during the last century by von Basch (4) who showed in animals and also in experimental models that pulmonary vascular congestion, presumably at the capillary level, tends to increase the volume of gas in the lungs. Recently, it was demonstrated in excised cats' lungs that the expansile force resulting from vascular congestion is greatest when the lungs are collapsed (5). Together, these findings might be taken as evidence underscoring the role of vascular distention in expanding the lungs at birth. Before this view is accepted, however, two facts must be established. One is that the increase in pulmonary blood flow precedes the first breath. This is contrary to the findings of Dawes in fetal lambs that pulmonary blood flow is increased only after inflation has occurred (6). The other is that the force developed through vascular filling or congestion is sufficiently large to cause air to enter the lung. The object of this study was to test this second possibility. Experiments were carried out to compare the distending forces produced by a wide range of pulmonary vascular pressures with the force required to inflate the lungs. Measurements were made on excised lungs of adult cats and dogs that were freed of gas to simulate their condition at birth, and on lungs of puppies and newborn infants that had not breathed.

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† Research Fellow, National Foundation for Infantile Paralysis, Inc.

METHODS

Excised lungs from four adult cats, one adult dog, five fetal puppies and two stillborn infants were used. The animal lungs were made gas-free by a modification of the technique of Coryllos and Birnbaum (7). The cats were anesthetized with 30 mg. per Kg. of pentobarbital (Nembutal®) [1] injected intraperitoneally; the dogs were given the same dose intravenously. Ninety-nine plus per cent of oxygen was used to ventilate the lungs through a tracheal cannula for about 15 minutes, after which the chest was opened and the trachea was occluded to permit absorption of the gas by the pulmonary circulation. Early in the period of gas-freeing 375 mg. of mepesulfate [2] (an anticoagulant), diluted with saline, was injected into the left ventricle. The heart and lungs were excised together. Cannulae were inserted into the pulmonary artery and left atrium; a clamp was put across the myocardium just below the mitral valve in order to close the pulmonary vascular system. Each cannula was connected to a dispensing burette and pressure transducer. To minimize the formation of pulmonary edema, 6 per cent dextran in Ringer's lactate solution was used to fill the blood vessels. Trypan blue was added to the solution to help detect leaks. A tracheal cannula was in-

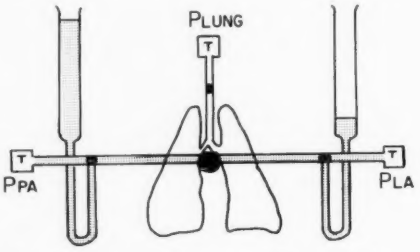

FIG. 1. SCHEMATIC DIAGRAM OF BURETTE, CANNULAE TO PULMONARY ARTERY AND LEFT ATRIUM, AND INTRATRACHEAL CANNULA

T indicates pressure transducer. Cannulae to pulmonary artery and left atrium are represented by black spot between the lungs.

[1] Abbott Laboratories.

[2] Hoffmann-La Roche, Inc.

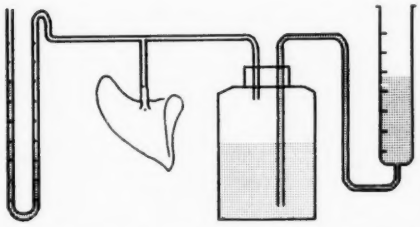

FIG. 2. DIAGRAM OF APPARATUS FOR MEASURING PRESSURE-VOLUME CHARACTERISTICS OF EXCISED LOBES

A correction for compression of gas within the bottle was made by clamping the trachea, elevating the burette, and subtracting the volume change recorded from the final readings with the lungs in the system.

serted close to the carina and connected to an inductance manometer. The gas volume of the cannula and tubing was kept small, ranging up to approximately 5 ml. The apparatus for this part of the experiment is shown schematically in Figure 1.

By varying the height of the dispensing burette leading to the vascular system, pressure could be raised to any level in the pulmonary artery and left atrium. Vascular congestion was maintained approximately 15 seconds, unless otherwise stated in the figure legends. Gas was not allowed to enter the lungs during these periods of vascular congestion so that changes only in airway pressure were possible. All pressures were recorded simultaneously on a direct-writing oscillograph.

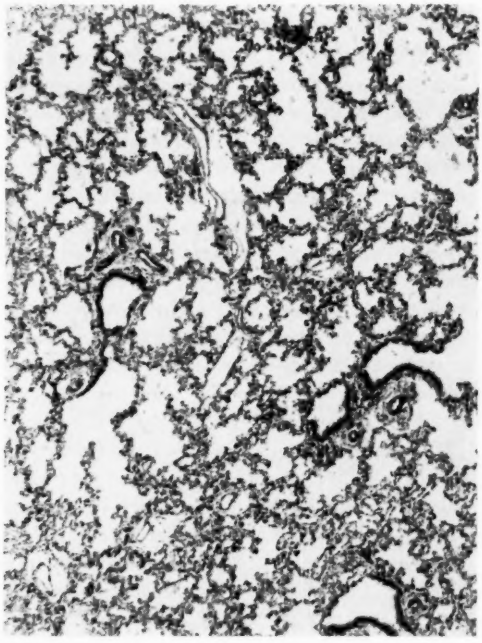

FIG. 3. SECTION OF RIGHT LUNG FROM 1,880 GM. STILLBORN ANENCEPHALIC MONSTER AFTER VASCULAR ENGORGEMENT AND FORMATION OF PULMONARY EDEMA

× 100. Hematoxylin and eosin.

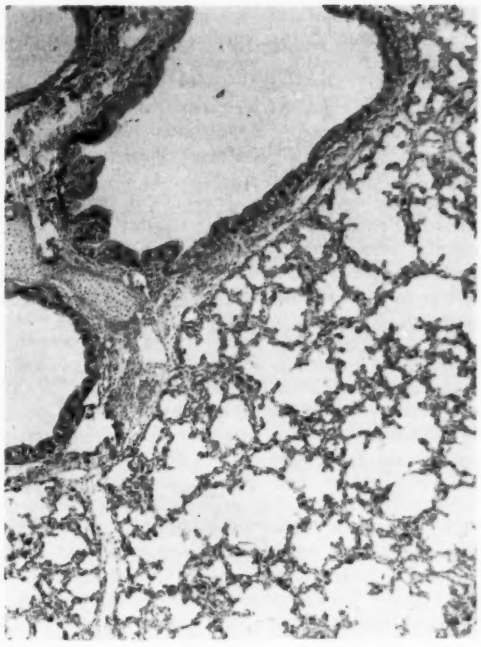

FIG. 4. SECTION OF LEFT LUNG FROM SAME INFANT AS IN FIGURE 3, AFTER INFLATION WITH AIR BY POSITIVE PRESSURE APPLIED TO TRACHEA

× 100. Hematoxylin and eosin.

One human left lung with a weight of 18 Gm. was studied four hours after death. It was obtained from a 1,500 Gm. anencephalic monster that made no respiratory movements but did have a pulsating heart for 40 minutes after delivery. The other human lung, from an 1,880 Gm. anencephalic monster, was studied 7.5 hours after stillbirth. The experimental procedure was the same as that outlined for the animals except that no anticoagulant was used, and the ductus arteriosus was tied off to produce a closed pulmonary vascular system. Histologic sections of the lungs from the 1,880 Gm. infant are shown in Figures 3 and 4.

The fetal puppies weighed from 140 to 240 Gm. and were obtained from two pregnant dogs estimated to be near term. One of the dogs was sacrificed by injecting Nembutal® intravenously, after which eight puppies were delivered as quickly as possible by cesarean section. Mepesulfate was injected into the fetal hearts which were beating at the time of delivery. The puppies did not breathe during the period of direct observation. The other dog was anesthetized with Nembutal® and the

lungs ventilated with 99 plus per cent oxygen through a tracheal cannula. An attempt was made to prevent intrauterine respiratory movements by injecting intravenously 0.3 Gm. morphine sulfate to sacrifice the animal. Before the puppies were removed from the uterus, the fetal tracheae were clamped; after delivery, the heart and lungs were excised and prepared in the manner already described; in addition, care was taken to occlude the foramen ovale with a clamp placed across the left atrium. Because of the extreme fragility of the cardiovascular tissue, leaks caused by damage to the tissue could not be avoided; as a consequence it was not possible to measure vascular volume, although vascular pressure was recorded accurately.

The preparation used to measure pressure-volume characteristics of excised lungs previously made gas-free is shown schematically in Figure 2. The lungs were obtained from five cats, 2 to 3.5 Kg. in weight, and five dogs, 14 to 18 Kg. in weight. Elevation of the dispensing burette shown on the right forced gas into the lungs. The difference between fluid levels in the burette and bottle represented the distending pressure for the lungs; this pressure was read from a water-filled manometer. Pressure was raised in steps of 2.5 cm. of water until a large portion of the lungs was inflated; thereafter, steps of 5 cm. of water were used until a peak level of 35 cm. of water (occasionally 30 cm. of water) was reached. The lungs were permitted to deflate in steps of 5 cm. of water. Two minutes were allowed for equilibration to occur before changes in volume were measured. Volume was read directly from the burette after correction was made for compression of the gas. Before and after each run, the volume of the lungs was measured by displacement of fluid.

To determine whether the distending pressure at which gas first entered the lungs could be influenced by the configuration of the lungs, these measurements were also made on individual lobes of dogs' lungs that were partially filled with saline. Two methods were used to instill fluid directly through the bronchial tree, and indirectly through the blood vessels by raising vascular pressure to levels at which "edema" fluid entered the air spaces. The amount of fluid added was roughly equal to the estimated functional residual capacity of the lobe, assuming that the distribution of the functional residual capacity among the lobes is proportional to their weight. Values for the functional residual capacity were based on the data of Mead and Collier (8).

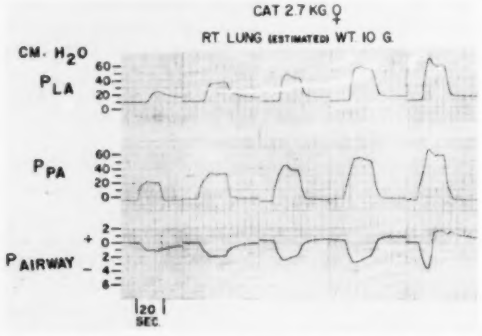

FIG. 5. PRESSURE TRACINGS FROM GAS-FREE CAT LOBE

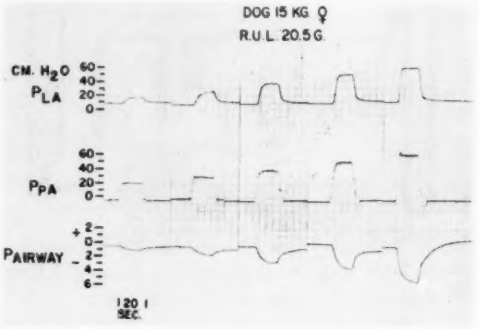

FIG. 6. PRESSURE TRACINGS FROM GAS-FREE DOG LOBE

RESULTS

Influence of acute pulmonary vascular congestion on airway pressure in gas-free excised lungs

The findings in adult cats' and dogs' lungs were similar. Typical tracings from two experiments are shown in Figures 5 and 6. The control pulmonary arterial pressure was set at about −4 cm. of water; in the experiments shown, left atrial pressure during the control period was set at 8 to 10 cm. of water which is within the normal range for the intact animal. In other experiments, control pressure in the left atrium was set at −4 cm. of water without any apparent difference in results. When arterial pressure was raised acutely to 20 cm. of water or more, pressure in the left atrium rose to nearly the same level within 15 seconds. Filling of the vascular system, judged from the distribution of the blue perfusate, appeared to occur throughout the lungs.

The range of pulmonary vascular pressure was from 20 to 80 cm. of water. Vascular volume and pressure showed a close correlation even though time was not allowed for complete equilibration to occur (Figure 7). At lower vascular pressures, almost all of the fluid was regained upon emptying the system. At vascular pressures of approximately 60 cm. of water or more, the recovery of fluid was incomplete, suggesting that edema or "leakage" had occurred.

In all experiments, the change in airway pres-

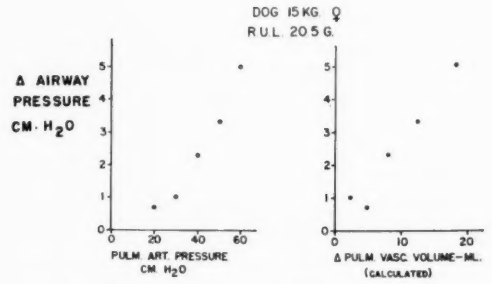

FIG. 7. THE RELATIONSHIP BETWEEN PULMONARY VASCULAR PRESSURE AND VOLUME, AND AIRWAY (TRANSPULMONARY) PRESSURE

sure followed within a few seconds the onset of congestion. The direction of change was toward subatmospheric; that is, the force which developed acted in the direction of expanding the lungs. (It will be recalled from the description of the preparation that gas was not permitted to enter the lungs.) The change in airway pressure correlated well with the level of vascular pressure, ranging from about 1 cm. of water at a vascular pressure of 20 cm. of water, up to nearly 5 cm. of water at vascular pressures of 50 to 70 cm. of water. The largest distending pressure developed in any lung in this series was 5 cm. of water.

At lower degrees of congestion, airway pressure approached equilibrium asymptotically and returned nearly to control level when congestion was reversed. At the highest vascular pressure, the response was biphasic: Initially, as before, airway pressure became subatmospheric, but it shortly reversed its direction and became positive relative to the atmosphere if the congestion was sustained long enough. This reversal appeared to correspond to formation of increasing amounts of edema. (The lungs were seen to increase significantly in volume.) It is noteworthy that, subsequent to this step, the response of airway pressure to lower levels of vascular pressure was significantly reduced (Figure 8).

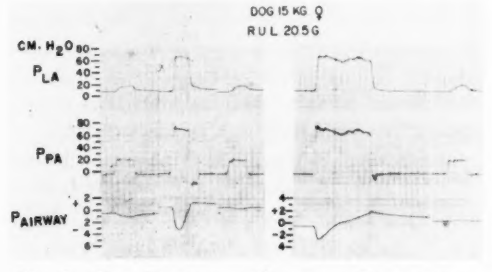

FIG. 8. THE EFFECT OF VASCULAR CONGESTION ON AIRWAY PRESSURE, SHOWING THE CHANGE IN SIGN IN THE AIRWAY DURING EDEMA FORMATION

In the human stillborn lung, pulmonary arterial pressure could not be transmitted to the left atrium. For this reason the procedure was reversed and left atrial pressure was elevated in an attempt to open the vascular system from the other direction. There was no retrograde transmission of pressure either, until atrial pressure reached 80 cm. of water; at this point arterial pressure rose suddenly to about 70 cm. of water. Associated with this extreme elevation of vascular pressure was a slight biphasic change in airway pressure; that is, there was a transient negative phase followed by a positive one (Figure 9).

The results of experiments on fetal puppies were less clear-cut, probably because of leakage out of the vascular system. The largest change induced in airway pressure during filling of the blood vessels was less than 1 cm. of water.

To ensure that pressure changes measured with the gas-filled recording system (tracheal cannula and pressure tubing) were a valid reflection of changes occurring at the alveolar level, two additional procedures were done. One was to place a gas-free cat's lung in an air-tight, stoppered glass jar; the lung was supported by its tracheal cannula leading through the stopper to a transducer; several sizes of cannulae and lengths of tubing

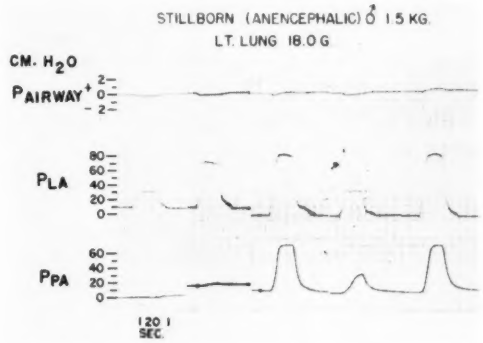

FIG. 9. PRESSURE TRACINGS OBTAINED DURING ELEVATION OF THE LEFT ATRIAL PRESSURE IN THE LUNGS OF A HUMAN STILLBORN

Note the very minimal changes in airway pressure accompanying vascular distention.

were used. Suction was then applied around the lungs in steps of 1 cm. of water up to a total of −6 cm. of water, while the pressure response inside the trachea was recorded. In general, about 80 per cent of the applied negative pressures was transmitted to the manometer; that is, a pressure of −5 cm. of water around the lungs was read as −4 cm. of water. The small variations of size of the cannulae and tubing used in the first experiments did not significantly affect this degree of transmission.

The second procedure was to repeat the experiment entirely under fluid (saline). In this system, zero reference pressure for both the lungs and blood vessels was the level of the saline bath. The assumption was made that in the absence of an air-fluid interface the changes in pressure at the alveolar level would be transmitted in total to the manometer. It was found that the magnitude of change in airway pressure induced by graded elevations of vascular pressure were approximately the same as those in the conventional preparation.

Volume-pressure characteristics of the gas-free and partially fluid-filled lungs

Typical examples of static volume-pressure characteristics of the separate lobes of a dog's lung are shown in Figure 10. To facilitate comparison of the lobes, intrapulmonary volume was expressed in ml. (gas, fluid, or both) per Gm. of tissue. In this preparation the right upper lobe was completely collapsed, the left lower lobe was partially filled with saline through its major bronchus, and the right middle lobe was partially filled with saline through its vascular system.

During inflation of the totally collapsed right upper lobe there was no appreciable increase in volume until a pressure exceeding 20 cm. of water was applied to the airway. Judging from the appearance of the pleural surface, filling was complete at 35 cm. of water. On the other hand, equivalent inflation of the two lobes that contained fluid occurred at pressures of only 12.5 and 15 cm. of water. These lobes also appeared completely inflated (there were no obviously collapsed units on the pleural surface) at a distending pressure of 35 cm. of water. The volume-pressure relationships of these lungs are illustrated in Figure 10.

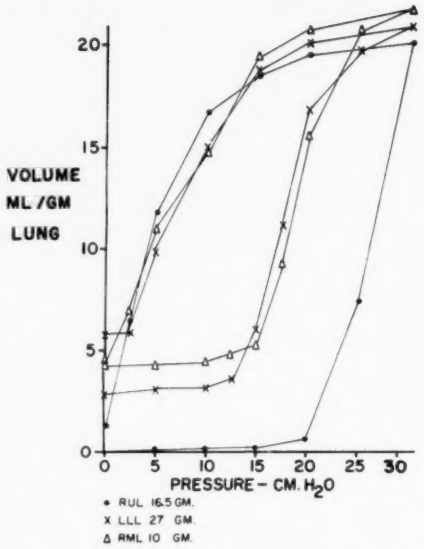

FIG. 10. PRESSURE-VOLUME CURVES OF EXCISED GAS-FREE DOG LUNGS

The inflation curves are on the right, deflation on the left. ● indicates gas- and fluid-free right upper lobe; ×, partially filled with saline through bronchus; and △, partially filled with saline through the vascular system.

During deflation, the curves for all three lobes were roughly similar except at zero airway pressure at which the volume was greater in the two lobes containing fluid; this occurrence of an increased volume at zero airway pressure in the presence of "edema" is typical for excised lobes (9).

DISCUSSION

Fluid was allowed to flow into, but not through, the vascular system from either side; approximately equal levels of pressure were therefore applied to all parts of the vascular system. We believe that the effect of congestion on the elastic behavior of the lungs is exerted largely at the level of the small vessels: the capillaries, and possibly venules, lying apposed to alveolar structures. In measurements during life the left atrial pressure which is generally accepted as a reliable index of capillary pressure should provide similar information about the degree of congestion.

In general, the changes produced in airway pressure acting to distend the lungs of the adult dogs and cats were roughly proportional to the

levels of vascular pressure that were applied. When the vascular pressure was raised to 30 cm. of water, an inflationary force of only 1 to 2 cm. of water was developed; at a vascular pressure of 40 cm. of water it rose to about 2.5 cm. of water; at levels of 60 to 80 cm. of water, the change in airway pressure never exceeded 5 cm. of water. Vascular pressures of 30 to 40 cm. of water have particular relevance, for when these levels are reached in the left atrium of living dogs, pulmonary edema forms (9). The higher vascular pressures in these experiments were induced to test the extreme situation, answering the question of how much effect on airway pressure is possible.

The relative lack of response among the lungs of stillborn infants and fetal dogs may have been due to technical shortcomings: clotted blood which prevented the transmission of pressure throughout the vessels (stillborn infants' lungs) and vascular leaks (fetal dogs' lungs). On the other hand, the presence of amniotic fluid or secretions in the lungs would tend to minimize the expansile effect of vascular congestion. That is, the distending force produced by a given degree of congestion is greatest when the lungs are at minimum volume, and becomes progressively smaller as the lungs expand (5). Evidence for this was found in the experiments in which vascular pressure was maintained at high levels for periods up to several minutes. Airway pressure, which at the outset fell to subatmospheric levels, gradually reversed its direction and finally became positive relative to atmospheric pressure (Figures 5, 6, 8, 9). Concurrently, the volume of the lungs, judging from their external appearance, increased significantly even though the trachea was occluded. This suggests that the increasing volume of the lungs did minimize the inflationary effect of vascular distention.

Fluid which may be present in the passages of the lungs prior to initial inflation by gas will have other implications as well. The results of these experiments suggest that if there is fluid equal in amount to half of the estimated functional residual capacity, inflation will proceed at lower distending pressure than if the lungs are totally collapsed (Figure 10). This effect may be due to changes in the configuration of the smaller units of the lung. Fluid, by enlarging the radii of curvature of the alveolar ducts, could facilitate expansion in accordance with the LaPlace relationship which states that the pressure in a tube is directly proportional to tension and inversely proportional to the radius of curvature ($P = T/r$). In the case of air entering a liquid-filled tube, the pressure required would be determined by the surface tension at the liquid-air interface, and the formula would then be $P = 2$ surface tension/radius (10).

The results of these experiments do not support the hypothesis developed by Jäykkä (1) and amplified by Bonham Carter (2) and Adams and co-workers (3), that the force developed by "capillary erection" at birth is a primary factor causing the first inflation of the lungs. Jäykkä's evidence for the hypothesis rested on a series of histological sections from lungs of stillborn infants and animals; vascular congestion had been produced following excision of the lungs. He found that the lungs were more evenly expanded if macrodex [3] was first injected through the pulmonary artery than if they were inflated by gas directly through the trachea. The conclusion was drawn that circulatory adjustments at birth are responsible for normal inflation. An alternative explanation for his histological findings is that macrodex which was injected into the pulmonary artery under a pressure of 80 mm. Hg entered the alveoli as edema fluid, while the particles of India ink used to stain the fluid remained in the capillaries. In the process of fixing the tissue macrodex may have been washed out, giving the illusion of alveoli inflated by gas. Figures 3 and 4 illustrate the difficulty of distinguishing between lungs that are filled with fluid or inflated with gas. Figure 3 shows a section from one lung of an 1,880 Gm. stillborn infant after edema was induced through the vascular system with macrodex stained with trypan blue. The other lung, which was inflated with gas through the trachea, is shown in Figure 4.

SUMMARY

The role that increased pulmonary blood flow at birth may play in expanding the lungs was studied by measuring the pressures developed in the lungs during acute vascular congestion. Measurements were made in excised lungs of four adult cats, one adult dog, five fetal dogs, and two stillborn infants. When the lungs were at minimum volume,

[3] Pharmacia Ltd., Sweden.

the effect of vascular distension was in the direction of facilitating expansion but it was not of sufficient magnitude to initiate it. When the lungs were partially distended by fluid, the expansile effect of vascular congestion was negligible.

The presence of a volume of fluid in the lungs, however, while minimizing the effects of vascular congestion, permitted expansion at a lower airway pressure than that required for lungs at minimal volume.

ACKNOWLEDGMENTS

We are grateful to Dr. James L. Whittenberger and Dr. Clement Smith for their advice and encouragement, and to Dr. Kurt Benirschke and the staff of the Department of Pathology of Boston Lying-In Hospital and Dr. John Craig of the Department of Pathology, Children's Hospital, for permission to study human lungs and for their help with the interpretation of histological sections.

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ANNOUNCEMENTS OF MEETINGS

The Fifty-First Annual Meeting of THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION will be held in Atlantic City, N. J., on Monday, May 4, 1959, at 9:00 A.M. at the Casino Theater on the Steel Pier.

The Sixteenth Annual Meeting of THE AMERICAN FEDERATION FOR CLINICAL RESEARCH will be held in Atlantic City, N. J., at the Casino Theater on the Steel Pier on Sunday, May 3, 1959, at 9:00 A.M.

THE ASSOCIATION OF AMERICAN PHYSICIANS will hold its Seventy-Second Annual Meeting in Altantic City, N. J., at the Casino Theater on the Steel Pier on Tuesday, May 5, 1959, at 9:30 A.M. and in the Vernon Room, Chalfonte-Haddon Hall, on Wednesday, May 6, 1959, at 9:30 A.M.

THE EFFECT OF A FALL IN FILTRATION RATE ON SOLUTE AND WATER EXCRETION IN HYDROPENIC MAN * †

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Considerable attention has been focused on the relation between the quantity of solute filtered and urine concentration (1–3). It has been shown that as the filtered solute load is increased in hydropenic subjects, the concentration of the urine falls despite continued antidiuretic activity (1–4). A major action of the antidiuretic hormone (ADH) is to render the distal tubule more permeable to water and the fluid therein progressively less dilute before it reaches the concentrating segment (5–7). An increment in the quantity of solute filtered and unreabsorbed in the proximal tubule increases the solute load reaching the distal tubule and thereby reduces the diffusion coefficient of the distal tubular water (8). Furthermore, the continued extraction of solute in the distal tubule increases the volume of water freed for back diffusion (7, 8). The existing levels of circulating ADH might then prove relatively less effective in promoting maximum back diffusion of distal tubular water so that the concentration of the fluid leaving the distal tubule would fall below serum osmolality (8). The diluting effect of an increase in the filtered solute load in hydropenic subjects might relate to this factor of less complete back diffusion of distal tubular water as well as to the increase in volume reaching the concentrating segment. To delineate further the influence of variations in the rate of filtration of solute on urine concentration, experiments were performed in man during which the glomerular filtration rate (GFR) was reduced in the face of intense antidiuretic activity. Such experiments were performed in subjects without renal disease and in patients with frank renal failure of diverse etiology.

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MATERIALS AND METHODS

Glomerular filtration rate was reduced for approximately two hours in 21 hydropenic subjects comprising three separate groups. Group Ia consisted of eight subjects with normal renal function, maintained on regular diets; Group Ib included six subjects with normal kidney function in whom the rate of sodium excretion had been markedly depressed. The latter group was prepared by the institution of a salt-free diet, containing 225 mg. of sodium, five days prior to the acute experiment. Forty-eight hours before the experiment, the oral administration of 9-alpha fluorohydrocortisone was started at a dosage level of 2 mg. every eight hours. Group II consisted of seven patients with frank renal failure of diverse etiologies.

Each patient received five units of Pitressin® Tannate in oil intramuscularly the night before, and was deprived of food and water for 16 hours prior to the experiment. The study was performed in the morning with the fasting patient remaining in bed. Catheterization was performed with a No. 18 multi-holed soft catheter or, in later experiments, a No. 18 malecott catheter. This latter type allows the catheter opening to lie flush with the internal urethral orifice, a position which permits an efficient bladder emptying without awkward and painful manipulation of the catheter. The overnight specimen was saved in a sterile syringe. A calibrated syringe was attached to the distal end of the catheter and the bladder was emptied every three minutes in order to estimate urine flow accurately at the bedside. The periods of very low urine flow could thus be terminated as soon as sufficient urine was collected.

A priming injection of inulin, sodium para-aminohippurate (PAH), and two units of Pitressin® was administered intravenously. This was followed by a constant infusion of these substances dissolved in Ringer's lactate in quantities adequate to measure GFR and effective renal plasma flow (ERPF), and to insure maximal antidiuretic activity. (Pitressin® was infused at a rate of 500 mU per hour.) This solution was administered intravenously with a Bowman infusion pump at a constant rate of 0.5 ml. per minute. After a 45 to 60 minute equilibration period, the bladder was emptied, rinsed with the overnight, inulin-free urine, and then with air. Thereafter three 30 minute control periods were completed and the bladder was emptied only with air.

The blood pressure was then reduced by the intravenous administration of 50 to 100 mg. of SC 1950

(1-ethyl-2,6-dimethylluptidine ethobromide),[1] a ganglionic blocking agent, and/or the application of venous tourniquets to the thighs. The drug alone was usually sufficient to lower the blood pressure in patients with any degree of hypertension; tourniquets and tilting were required in addition in the normotensive subjects. The hypotension was maintained for about two hours during which 3 to 4 more urine collection periods of approximately 45 minutes each were completed. Blood specimens were collected at appropriate intervals throughout the control and hypotensive periods. Each blood and urine sample was analyzed for total osmolality and inulin, PAH, sodium, potassium, chloride and urea concentrations. Urine pH and CO_2 content were determined in several experiments. Clearances were calculated by standard methods.

In the subjects in Group Ib, the maximum osmolality of the urine was measured before the salt-free diet was instituted. As in the preparation for an experiment, five units of Pitressin® in oil was administered the night before and the patient was subsequently deprived of all food and water for 16 to 18 hours. The following morning urine specimens were collected at 7 and 8 a.m. for the measurement of total osmolality.

Three normal subjects were exposed to identical experimental protocols but failed to sustain a measurable fall in glomerular filtration rate. This group, therefore, served as a control for the three experimental groups studied in which each subject underwent a fall in glomerular filtration rate of at least 20 per cent.

Osmolality was measured with a Bowman-Aminco freezing point depression apparatus (9). Sodium and potassium concentrations were determined with an internally standardized flame photometer, chloride by the Whitehorn modification of the Volhard method (10), urea by the micro-method of Steinitz using Conway units (11), inulin by Schreiner's method (12), and PAH by the method of Smith and co-workers (13).

RESULTS

During the three control periods in all of the experimental subjects, the urine osmolality tended to rise slightly. The mean increment per control period equalled 10 mOsm. per L. but the maximum increase in urine osmolality throughout the prehypotensive periods did not exceed 40 mOsm. per L. Accordingly, the separate control osmolalities, as well as the other separate control modalities measured, were averaged and presented as one control figure in Tables I through IV. In the three subjects in whom the GFR was not reduced 10 per cent or more, the urine osmolality continued to rise slightly at a rate not appreciably different from that noted during the prehypotensive

[1] This drug was generously supplied by the G. D. Searle Co., Chicago, Ill., through Dr. Irwin C. Winter, Clinical Director.

TABLE I

The effect of typical experimental protocol in subjects in whom filtration rate was not reduced *

Patient (Age, sex, S.A.† diagnosis)	Period min.	Urine flow ml./min.	Inulin [V/P]† ml./min.	Osmolality U† mOsm./Kg.	Osmolality UV† μOsm./min.	Sodium U mEq./L.	Sodium UV μEq./min.	Chloride U mEq./L.	Chloride UV μEq./min.	Potassium U mEq./L.	Potassium UV μEq./min.	Urea U mM/L.	Urea UV μM/min.
C. M. (22, F, 1.30 M.², pneumonia)	95	0.76	88	787	598	176	134	126	96	172	131	117	88
	36	0.58	80	803	466	177	103	134	78	192	111	125	73
	26	0.67	78	820	549	186	125	116	79	181	121	126	84
	24	0.52	82	836	435	195	102	143	74	176	92	136	70
A. J. (26, F, 1.37 M.², mononucleosis)	92	0.72	107	819	588	98	70	122	88	162	117	124	89
	41	0.61	102	838	511	90	55	107	65	171	104	118	62
	18	0.58	98	846	490	110	64	115	67	180	104	121	70
	35	0.64	104	851	549	96	61	104	67	173	111	131	84
K. M. (34, F, 1.62 M.², lymphoma)	86	0.57	134	872	497	110	63	137	78	187	107	114	65
	37	0.61	130	903	551	104	63	120	73	190	116	120	73
	51	0.52	126	921	479	93	48	114	59	204	106	122	63
	42	0.49	122	932	457	100	49	123	60	198	97	128	63

* The top row of data for each subject represents the average of three control periods.
† Abbreviations are as follows: S.A., surface area; UV/P, clearance; U, urinary concentration; V, urinary flow.

TABLE II

The effect of a fall in glomerular filtration rate (GFR) on solute and water excretion (subjects on normal salt intake) *

Patient (Age, sex, S.A., diagnosis)	Period *min.*	Urine flow *ml./min.*	Inulin UV/P *ml./min.*	Osmolality U *mOsm./Kg.*	Osmolality UV *μOsm./min.*	Sodium U *mEq./L.*	Sodium UV *μEq./min.*	Chloride U *mEq./L.*	Chloride UV *μEq./min.*	Potassium U *mEq./L.*	Potassium UV *μEq./min.*	Urea U *mM/L.*	Urea UV *μM/min.*	Urea/Cinulin
H. L.	64	0.35	131	985	345	131	46	151	53	157	55	141	50	0.10
(26, F, 1.49 M.²,	50	0.24	86	953	229	108	26	121	29	182	44	137	33	0.09
pulm. TB)	29	0.17	68	883	150	76	13	73	12	184	31	118	20	0.08
	39	0.24	89	923	222	107	26	106	26	177	43	140	34	0.10
L. J.	90	0.65	90	820	583	100	65	134	87	186	121	60	39	0.11
(55, F, 1.48 M.²,	28	0.29	50	852	241	103	29	112	32	204	58	48	14	0.07
pulm. TB)	52	0.13	54	702	91	43	6	56	7	188	24	44	6	0.02
	45	0.20	70	830	176	41	8	57	11	228	46	55	11	0.04
G. T.	56	0.57	112	788	449	186	106	145	83	160	91	93	53	0.10
(37, F, 1.67 M.²,	43	0.30	85	808	243	220	66	146	44	153	46	100	30	0.07
cystocele)	41	0.28	70	802	225	181	51	145	41	198	56	93	26	0.07
	46	0.22	79	751	165	96	21	118	26	281	61	79	17	0.04
	45	0.18	69	807	145	69	14	105	19	301	54	86	15	0.05
I. M.	98	0.27	68	1,027	277	141	38	133	36	105	28	245	66	0.21
(41, F, 1.46 M.²,	40	0.22	55	1,029	226	150	33	143	31	97	21	271	60	0.24
bronchiectasis)	45	0.13	43	972	126	110	15	105	14	117	16	186	24	0.13
	52	0.21	52	951	200	113	24	74	15	120	25	205	43	0.19
J. R.	87	1.05	88	562	590	151	159	159	170	94	99	88	92	0.26
(59, F, 1.47 M.²,	53	0.73	39	543	396	149	109	153	117	83	61	90	66	0.42
Hodgkin's dis.)	25	0.26	34	523	136	76	20	103	27	119	31	88	23	0.51
	156	0.07	20	456	32	30	2	53	4	131	9	61	4	0.05
	25	0.24	71	416	100	28	7	45	11	107	26	51	12	0.04
F. P.	57	1.16	100	478	554	68	79	98	114	110	128	86	100	0.16
(30, M, 1.62 M.²,	33	1.02	93	526	537	69	70	101	103	113	115	89	91	0.16
schistosomiasis)	24	0.31	57	468	145	20	6	72	22	154	48	82	25	0.07
	25	0.28	72	461	129	6	2	51	14	155	43	67	19	0.04
	25	0.34	84	536	182	10	3	48	16	166	56	103	35	0.07
C. R.	58	0.85	133	920	782	134	114	144	122	115	98	125	106	0.26
(36, F, 2.08 M.²,	29	0.78	103	727	567	140	109	128	100	118	92	127	99	0.32
pneumonia)	28	0.27	69	776	210	124	33	49	13	144	39	121	33	0.16
	59	0.12	70	774	93	63	8	17	2	181	22	105	13	0.06
	36	0.25	114	922	231	132	33	40	10	146	37	127	32	0.09
C. T.	75	0.34	95	947	322	20	7	115	39	298	101	205	70	0.15
(29, F, 1.47 M.²,	31	0.36	91	956	344	55	20	148	53	301	108	211	76	0.17
duod. ulcer;	71	0.09	50	837	75	14	1	76	7	294	26	125	11	0.04
milk diet)	45	0.17	83	1,013	172	12	2	79	13	354	60	213	36	0.09
	38	0.22	86	1,134	249	36	8	100	22	331	73	277	61	0.14

* The top row of data for each subject represents the average of three control periods.

TABLE III

The effect of a fall in glomerular filtration rate (GFR) on solute and water excretion (subjects on a low-salt diet) *

Patient (Age, sex, S.A., diagnosis)	Period *min.*	Urine flow *ml./min.*	Inulin UV/P *ml./min.*	Osmolality U *mOsm./Kg.*	Osmolality UV *μOsm./min.*	Sodium U *mEq./L.*	Sodium UV *μEq./min.*	Chloride U *mEq./L.*	Chloride UV *μEq./min.*	Potassium U *mEq./L.*	Potassium UV *μEq./min.*	Urea U *mM/L.*	Urea UV *μM/min.*	C_{urea}/C_{inulin}
				(1,023)										
M. R.	114	0.12	57	975	117	25	3.0	57	7.0	194	23	220	27	0.09
(26, F, 1.30 M.²,	58	0.10	52	934	93	23	2.3	57	6.0	184	18	242	24	0.09
rheum. arth.)	59	0.09	41	921	78	19	1.6	26	2.0	141	12	188	16	0.08
	58	0.13	63	917	118	14	1.8	33	4.0	138	18	214	28	0.09
				(745)										
A. P.	71	0.32	65	793	254	27	8.6	26	8.4	126	40	178	57	0.18
(62, F, 1.35 M.²,	30	0.30	63	797	239	31	9.4	31	9.2	91	27	170	51	0.16
irritable colon)	26	0.25	60	813	203	22	6.4	25	6.2	131	33	169	42	0.12
	41	0.16	46	770	123	16	2.6	20	3.2	137	22	164	26	0.08
				(1,051)										
M. B.	85	0.15	100	1,073	161	9	14.0	54	8.0	132	20	300	45	0.11
(16, F, 1.40 M.²,	42	0.20	73	1,007	200	21	4.0	68	14.0	129	26	276	55	0.19
pneumonia)	43	0.13	59	1,036	135	11	1.4	52	7.0	163	21	263	34	0.14
	54	0.11	64	1,136	125	9	0.9	56	6.0	191	21	283	31	0.12
				(1,087)										
D. C.	82	0.15	133	1,012	152	10	2.0	50	8.0	195	29	306	46	0.09
(15, F, 1.52 M.²,	47	0.19	124	1,068	203	13	2.0	59	11.0	193	37	322	61	0.12
dysmenorrhea)	55	0.12	82	908	109	9	1.0	50	6.0	155	19	223	27	0.08
	43	0.14	86	923	129	7	1.0	49	7.0	198	27	243	34	0.10
				(912)										
A. B.	90	0.23	51	1,110	255	33	8.0	57	13.0	133	31	278	64	0.25
(76, F, 1.74 M.²,	61	0.07	21	993	70	33	2.0	57	4.0	119	8	260	18	0.17
pneumonia)	52	0.13	43	968	126	19	2.0	37	5.0	131	17	236	31	0.14
	47	0.16	40	1,072	172	26	4.0	53	8.0	131	21	248	40	0.20
				(984)										
R. S.	67	0.33	110	754	249	5	1.7	75	24.8	213	70	186	59	0.08
(31, F, 1.53 M.²,	34	0.24	64	646	155	4	1.0	78	18.4	199	48	164	39	0.09
irritable colon)	54	0.11	56	607	67	6	0.7	68	7.6	178	20	146	16	0.04
	41	0.17	92	832	141	8	1.4	68	11.5	230	39	181	31	0.05
	30	0.27	96	933	252	8	2.2	67	17.9	280	76	171	46	0.05

* The top row of data for each subject represents the average of three control periods. The osmolality in parentheses represents the maximum urine concentration prior to the institution of the salt-free diet.

TABLE IV

The effect of a fall in glomerular filtration rate (GFR) on solute and water excretion (subjects with renal failure) *

Patient (Age, sex, S.A., diagnosis)	Period *min.*	Urine flow *ml./min.*	Inulin UV/P *ml./min.*	Osmolality U *mOsm./Kg.*	Osmolality UV *μOsm./min.*	Sodium U *mEq./L.*	Sodium UV *μEq./min.*	Chloride U *mEq./L.*	Chloride UV *μEq./min.*	Filtered Cl excret. *%*	Potassium U *mEq./L.*	Potassium UV *μEq./min.*	Urea U *mM/L.*	Urea UV *μM/min.*	C_{urea}/C_{inulin}
S. G.	53	1.05	4.1	238	250	71	75.0	66	69	18.0	21	22	71	75	0.92
(69, F, 1.56 M.²,	21	1.10	4.0	247	272	70	77.0	67	74	19.0	22	24	78	86	1.06
chr. glomneph.)	32	0.31	1.8	266	82	50	16.0	57	18	10.0	34	11	97	30	0.83
H. H.	60	1.15	6.3	282	324	61	70.0	67	77	11.0	32	37	81	93	0.59
(63, F, 1.58 M.²,	23	0.22	1.1	282	62	50	11.0	58	13	11.0	35	8	96	21	0.76
chr. glomneph.)	31	0.24	2.3	294	71	25	6.0	20	5	2.1	44	11	104	25	0.43
	32	0.31	3.2	297	92	4	1.2	13	4	1.3	54	17	126	29	0.47
E. A.	67	0.55	5.6	370	204	28	15.0	28	15	2.7	45	25	130	72	0.47
(47, F, 1.62 M.²,	34	0.15	1.5	382	57	28	4.0	27	4	2.5	46	7	159	24	0.59
chr. glomneph.)	27	0.22	2.7	378	83	21	5.0	21	5	1.6	53	12	136	30	0.41
	17	0.62	7.6	378	234	16	10.0	18	11	1.4	57	35	117	73	0.35
F. S.	69	0.43	8.8	434	187	29	12.0	39	17	1.9	59	25	132	57	0.37
(65, F, 1.53 M.²,	31	0.31	6.9	432	135	21	6.5	29	9	1.2	61	19	190	59	0.49
chr. glomneph.)	105	0.14	3.9	350	49	13	1.8	23	3	0.6	73	10	146	20	0.31
	50	0.15	4.7	348	52	12	1.8	22	3	0.7	74	11	148	22	0.28
	54	0.19	5.6	357	68	13	2.5	21	4	0.7	76	14	150	29	0.29
M. W.	61	0.91	23.0	407	370	85	77.0	90	82	3.4	34	31	124	113	0.55
(57, F, 1.70 M.²,	28	0.36	9.0	406	146	74	27.0	87	31	3.0	36	13	143	51	0.65
diab. glomscler.)	81	0.09	6.0	322	29	24	2.0	36	3	0.5	51	5	78	7	0.15
	25	0.22	12.0	337	74	18	4.0	30	7	0.5	60	13	104	23	0.22
	39	0.21	12.0	346	72	22	5.0	33	7	0.5	54	11	116	24	0.23
S. P.	46	2.85	24.0	334	952	61	174.0	87	248	10.4	39	111	68	194	0.58
(68, M, 1.50 M.²,	21	0.79	5.0	328	259	59	47.0	83	66	13.0	40	32	65	51	0.73
art. nephscler.)	23	0.30	3.0	362	109	40	12.0	54	16	5.4	43	13	76	23	0.54
	9	1.11	12.0	351	390	35	39.0	51	57	4.5	44	49	82	91	0.52
	28	2.43	37.0	441	1,076	15	36.0	38	92	2.5	46	112	119	289	0.56
C. R.	56	1.06	21.0	378	402	45	48.0	75	80	4.0	56	59	85	90	0.54
(33, F, 1.46 M.²,	28	0.77	17.0	384	296	40	31.0	67	52	2.9	58	45	95	73	0.54
chr. pyelo.)	58	0.15	12.0	403	60	15	2.0	28	4	0.3	54	8	69	10	0.10
	31	0.21	19.0	379	79	12	3.0	14	3	0.2	48	10	48	10	0.07
	32	0.27	21.0	374	101	14	4.0	14	4	0.1	51	14	54	15	0.09

* The top row of data for each subject represents the average of three control periods.

periods (Table I). In these subjects, no appreciable changes occurred in the rate of urea or electrolyte excretion.

In eight normal subjects [2] maintained on a regular salt intake, the hypotension produced a fall in filtration rate (Table II). The maximum fall, generally achieved during the second experimental period, averaged 47 per cent and ranged between 37 and 77 per cent. In association with this change, there was a consistent decrease in urine flow from an average of 0.68 ml. per minute to 0.14 ml. per minute. In each subject in whom the filtration rate was reduced, there was a consistent drop in urine osmolality averaging 91 mOsm. per L. or approximately 11 per cent of control solute concentrations (Table II). These falls in total solute concentration occurred regardless of the degree of urine hypertonicity achieved during the control period. In four of the eight subjects a slight increase in urine osmolality was detected in the first experimental period prior to the maximum fall in glomerular filtration rate. The total rate of solute excretion declined proportionately more than the coincident decrease in filtration rate (Table II). Sodium and chloride concentrations in the urine fell markedly, averaging 55 and 46 per cent, respectively (Table II). In association with the pronounced decrease in urine flow, the rate of sodium and chloride excretion fell almost 90 per cent. Simultaneously, there occurred a consistent increase in the potassium concentration of the urine ranging from 13 to 87 per cent and averaging 39 per cent (Table II). The rate of potassium excretion, however, fell slightly more than 60 per cent or considerably less than the coincident decrement in chloride excretion. In four subjects, in whom it was measured, the reduction in filtration rate produced a decrease in urine pH and CO_2 content.

During the period of reduced filtration rate, the urea concentration fell consistently, the decline ranging between 14 and 80 mOsm. per L. and averaging 34 mOsm. per L. (Table II). Accordingly there occurred a considerable fall in the rate of urea excretion and in the ratios of the urea to inulin clearance. This latter ratio diminished from a control average of 0.17 to 0.06 (Table II).

[2] "Normal" is used only to denote the absence of any clinical or hemodynamic evidence of renal disease.

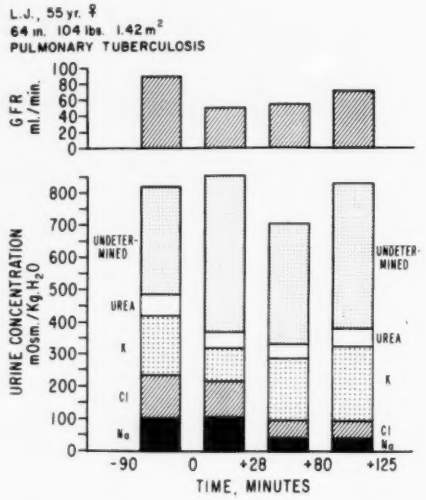

FIG. 1. CHANGES IN URINE SOLUTE CONCENTRATION FOLLOWING A FALL IN FILTRATION RATE IN HYDROPENIC SUBJECTS WITHOUT RENAL DISEASE

The typical change in the solute concentration of the urine effected by a fall in filtration rate is presented in Figure 1. There occurred a considerable fall in salt concentration, a lesser decrease in the fraction composed of urea and a moderate rise in potassium concentration and in the concentration of the nonmeasured solute.

In the six subjects maintained on salt-free diets and treated with salt-retaining hormone, the hypotension produced a fall in filtration rate averaging 41 per cent and ranging from 27 to 59 per cent (Table III). Compared with Group Ia, the control rates of urine flow were considerably lower, averaging 0.21 ml. per minute and falling to 0.11 ml. per minute (Table III). As in the previous experimental group there was a consistent fall in urine osmolality averaging 62 mOsm. per L. or 9 per cent of the control solute concentration (Table III). The control maximum urine osmolalities obtained on the experimental day did not differ appreciably from similar measurements performed prior to the institution of the special diet and hormone therapy (Table III). The proportional changes in salt concentration and the rate of salt excretion were similar to those observed in Group Ia but the control rates of salt excretion were so low that the absolute changes tended to be small. Changes in potassium excretion were similar to

those noted in Group Ia. As in Group Ia there occurred a consistent fall in urine urea concentration averaging 26 mOsm. per L. (Table III). However, the decrease in urea to inulin clearance ratio was not as marked as those reported above (Table III).

In seven patients with renal failure, comparable periods of experimental hypotension produced a maximum fall in filtration rate averaging 67 per cent (Table IV). The control rates of urine flow were considerably higher than that in the previous groups, averaging 1.14 ml. per minute and falling to 0.19 ml. per minute. The changes in urinary osmolality differed from those observed in the previous groups. In five patients, there was a slight increase in urine osmolality whereas two showed the falls characteristic of the normal subjects (Table IV). Compared to the normal groups a much higher percentage of the filtered solute and chloride was excreted in the urine (averages of 11 and 6 per cent, respectively). After the filtration rate was reduced, there occurred a very conspicuous fall in the concentration and rate of excretion of salt (Table IV) so that the percentage of the filtered salt load excreted in the urine more closely approached this comparable fraction observed during the control periods in the normal subjects. The changes in the concentration and rate of excretion of potassium were very similar to those observed in the normal groups (Table IV).

The changes in urea concentration corresponded to those of total solute concentration, five patients showing a slight increase in urea concentration and two a moderate decline (Table IV). The ratio between the urea and inulin clearance in this group far exceeded that noted in the normal groups and averaged 0.57 (Table III). Following the fall in filtration rate, this ratio decreased considerably but hardly reached the control ratio observed in the normal subjects.

Typical changes in the solute composition of the urine in a uremic subject in whom filtration rate was reduced are presented in Figure 2. The slight increase in total solute concentration is associated with a slight increase in urea and potassium concentration but a marked fall in salt concentration.

In all three groups, the proportionate fall in PAH clearance was similar to that of the inulin

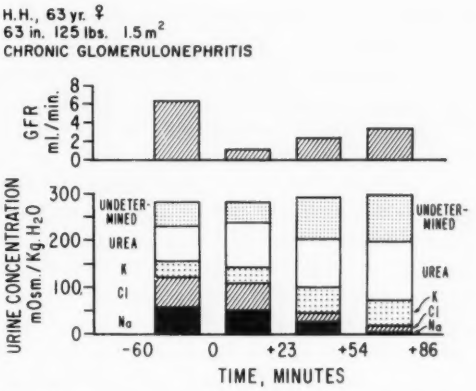

FIG. 2. CHANGES IN URINE SOLUTE CONCENTRATION FOLLOWING A FALL IN FILTRATION RATE IN HYDROPENIC SUBJECTS WITH FRANK RENAL FAILURE

clearance so that the filtration fraction remained unchanged.

In Groups Ia and Ib the filtration rate tended to return toward control values in the final experimental period (Tables I and II). This increase was often associated with a substantial rise in the urine osmolality toward, and even above, the control concentrations (Tables II and III).

DISCUSSION

On the basis of the data presented, it seems evident that 14 subjects in the normal group, both on the salt-free and on the regular diets, sustained a diminution in GFR ranging between 30 and 50 per cent which persisted for at least one hour. Although the absolute GFR measurements in the subjects with frank renal failure may have limited value, it appears that these subjects underwent a percentile fall in filtration rate at least as great as that recorded in the normal subjects.

Because of the low rates of urine flow, particularly during the hypotensive periods, the measured urinary concentrations represent parameters from urine that was formed some time prior to collection. Consequently, the observed osmolalities probably correspond best to the filtration rate recorded during the preceding period. Throughout the control periods, the urine osmolality tended to rise slightly because of continuing fluid deprivation and Pitressin® administration. This small but progressive rise in urine osmolality was particularly evident in those subjects in whom the ex-

perimental conditions failed to induce a fall in GFR (Table I). This gradual increment in urine concentration prior to the fall in filtration rate tends to underscore the consistent fall in urine osmolality noted in those normal subjects in whom the GFR was reduced.

According to present concepts of renal function, a fall in filtration rate in hydropenic, Pitressin®-infused subjects should enhance maximum back diffusion of water from the distal convoluted tubule assuring a peak concentration equal to serum osmolality (7, 8, 14–16). Furthermore, this stimulus appreciably reduces the water and solute load reaching the collecting duct. The continued extraction of solute-free water at this concentrating segment might therefore be expected to increase the urine osmolality to its maximum. Such an increase was observed in the first experimental period of several normal subjects (Tables II and III, Figure 3).[3] That these experimental conditions ultimately effected a fall in urine osmolality in all the normal subjects in whom the GFR was reduced (Figure 3) suggests that a considerable reduction in the rate of filtration of solute diminished the efficiency of the concentrating segment.

Considerable evidence suggests that urine is concentrated in the collecting duct by the flow of tubular fluid past a hypertonic medullary interstitial fluid (15, 16). According to this view, solute, particularly sodium, is actively transferred into this site by a process, the exact mechanism of which remains uncertain (15–17). Of equal relevance to this hypothesis is the presence in the medulla of a rich hairpin capillary plexus which creates a counter-current circulation tending to trap whatever solute is deposited in this site (16, 17).

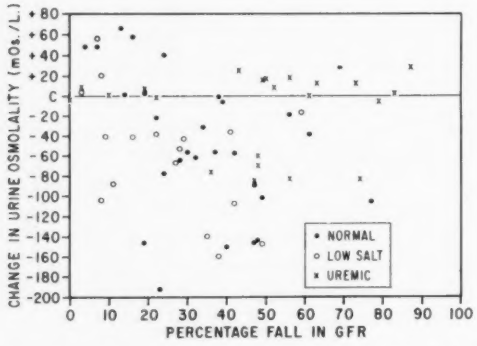

FIG. 3. PERCENTAGE CHANGES IN FILTRATION RATE PLOTTED AGAINST ABSOLUTE CHANGE IN TOTAL URINE SOLUTE CONCENTRATION IN ALL THREE GROUPS STUDIED

[3] In fact, this inconstant increase in urine osmolality may simply reflect the continuing slight increase in urine osmolality noted during the control periods (Table I).

The reduction in renal and presumably medullary blood flow produced in these experiments might be the factor responsible for reducing the concentration of the urine. However, if medullary ischemia does prevent the elaboration of a maximally concentrated urine, it would be difficult to explain the slight increase in urine solute concentration noted in the majority of the uremic subjects. Furthermore this hypothesis would not concur with the reported increase in urine concentration produced by a comparable stimulus in dog and man with diabetes insipidus (6, 7, 18). In terms of the counter-current hypothesis, a reduction in medullary flow should enhance the solute trapping effect and thereby increase the quantity of solute confined within the medulla and the osmolality of the urine (17).

It is conceivable that a fall in filtered load so reduces the quantity of sodium escaping absorption by the proximal tubule that the amount remaining for transfer into the medulla is too small to achieve maximum urine concentration. In the subjects in Group Ib, the combination of dietary sodium restriction and the administration of a potent sodium retaining hormone probably reduced the quantity of sodium escaping proximal reabsorption. Nevertheless, these subjects were able to concentrate their urine to a degree comparable to that achieved prior to sodium restriction. When the filtration rate was reduced in these patients, further depressing the flow of sodium beyond the proximal tubule, the falls in urine osmolality were not significantly different from those observed in the untreated subjects. This finding suggests that the decrease in filtration rate did not appreciably limit the quantity of sodium available for transfer into the medulla. These considerations tend to rule out renal ischemia and a diminished filtered sodium load as the explanation for the observed fall in urine osmolality in these experiments.

Another solute which accumulates in the hypertonic medulla is urea. Presumably urea enters this site by a process of passive diffusion, the rate

of which is dependent upon the concentration gradient between fluid in the collecting tubule and that in the medulla (17). This gradient is produced, in large part, by the rapid outward diffusion of water as the tubular fluid enters the area of medullary hypertonicity. A fall in filtration rate with continued back diffusion of urea throughout the length of the tubule markedly reduces the clearance ratios of urea to inulin and, accordingly, the quantity of urea entering the concentrating segment. The rate of back diffusion of urea at this site would therefore be considerably reduced and the effect on total medullary osmolality would be diminished. As the urea concentration at this medullary site falls, the back diffusion of whatever tubular urea reaches it would be more complete. Because the rate of total solute and urea entering this segment is much reduced, the continued back diffusion of urea at the concentrating segment or any more distal site would effect a more conspicuous fall in total solute concentration of the urine.[4] The combined effect of a fall in the rate of back diffusion of urea on total medullary concentration and of the continuing back diffusion of urea at, or distal to, the concentrating segment would explain the net fall in urine solute and urea concentration observed in normal subjects.

Many reports in the literature attest to the role of high protein and urea loads in producing maximum concentration of the urine (20–22). The fall in urine osmolality effected by protein and urea deprivation may to some extent relate to the reduced urea loads available for back diffusion in the collecting duct.

This hypothesis, proposed to explain the changes in the normal subject, is in accord with the opposite changes in urine solute concentration noted in the patients with renal failure. The relatively larger quantities of filtered urea excreted, as evident in the high urea to inulin clearance ratios, tend to impose an osmotic diuresis on the operating nephrons. This circumstance assures that a relatively good urea load may reach the collecting duct after the fall in filtration rate. The effects of the fall in urea load on the concentrating segment would therefore be muted. Further, the osmotic diuresis in the uremic patient may, even with a Pitressin® infusion, prevent maximum back diffusion of distal tubular water (8). The increment in the distal tubular fluid concentration effected by a reduced rate of flow might tend to obscure the simultaneous reduction in the efficiency of the concentrating segment. This dual effect may resolve the discrepancy between a net increase in urine concentration produced by a fall in filtration rate in diabetes insipidus dogs with the failure in these same experiments to produce a maximally concentrated urine (7).

It has been reported that in the dog comparable experiments produced similar but more marked falls in urine concentration (23). These experiments differ from those reported here in that much more sizeable reductions in filtration rate were attainable in the experimental animal.

The fall in urine concentration recorded in these experiments seems best explained by, and therefore supports, the hypothesis that urine is concentrated by the flow of tubular fluid past an area of medullary hypertonicity. However, these data may also be explained in other terms. It is conceivable, for example, that the fall in urine solute concentration might be related to a reduction in blood supply to separate populations of nephrons. The reduced renal perfusion precipitated by experimental hypotension may eliminate filtration in those nephrons dipping deeply into the medulla and supply blood only to those with shorter tu-

[4] Since these experiments were completed, Levinsky and Berliner have shown that a small concentrated volume of urine left in the bladder for 30 minutes may lose a considerable fraction of its urea, presumably, via a process of passive back diffusion (19). While an undetermined fraction of the total fall in urine osmolality noted in these experiments may result from the continuing back diffusion of urea at the concentrating segment or any site distal thereto, it is difficult to ascribe the entire drop in osmolality to the back diffusion of urea in the bladder *per se*. During the hypotensive period, the bladder was constantly drained and emptied so that each period could be terminated as soon as 6 ml. of urine was obtained. Consequently, the urine was not permitted to lie in contact with the bladder mucosa for more than five minutes. Furthermore, in the uremic subjects where the same back diffusion of bladder urea would be expected, the urine urea concentration rose during the period of hypotension. Regardless of the length of the period of urine collection, no fall in urine osmolality or urea concentration occurred during any control period or any experimental period not preceded by a considerable fall in glomerular filtration rate. Finally, in each experimental period in which a fall in urine osmolality occurred, this decrease exceeded the simultaneous drop in urinary concentration of urea.

bules producing a more dilute urine. It has also been suggested that the remaining nephrons in operation may be exposed to an increased filtered load and act as if under the influence of an osmotic diuresis with a reduction in urine osmolality (24).

The fact that considerable falls in filtration rate may occur without any measurable fall in glucose Tm argues against the possibility that any appreciable number of nephrons have been cut out of circulation (25). According to this alternate hypothesis, the fall of 90 to 95 per cent in salt excretion associated with a 50 per cent fall in filtration rate would demand that half of the operating nephrons normally excrete only 5 to 10 per cent of the salt. This seems unlikely, particularly if the nephrons remaining in operation represent those with the shortest tubules and least opportunity for salt reabsorption. That the operating nephrons are subject to a solute diuresis is not borne out by the proportionately greater fall in solute excretion than in filtration rate (Tables II, III). It is therefore difficult to explain these observed changes in solute and water excretion on the basis of alterations in the nephron population remaining in operation. It seems instead that some reduction in filtration rate was produced in virtually all glomeruli—an assumption inherent in the original hypothesis.

Proportionately large falls in salt excretion following a reduction in the filtration rate of normal man and dog have been previously recorded (26–28). This conspicuous change has been attributed to the prolonged contact of the glomerular filtrate with the salt absorbing tubules. The lesser changes in the rate of potassium excretion has likewise been emphasized. It has been argued that the rate of potassium excretion plunges only when the quantity of sodium available to the distal or exchanging segment begins to fall appreciably (29). The data in the normal subjects following a fall in filtration rate are in accord with these previous observations and hypotheses.

Similar changes in salt and potassium excretion following a comparable stimulus in uremic subjects suggest that the operating nephrons retain the capacity for considerable salt absorption. The failure of the uremic subjects to absorb comparable fractions of the filtered load therefore seems to stem from an osmotic diuresis imposed by the urea load or by a glomerular preponderance in the remaining nephrons (30). The elimination of the glomerular-tubular imbalance in these subjects then leads to a more complete reabsorption of filtered salt similar to that noted in the normals.

SUMMARY

1. Glomerular filtration rate was reduced at least 50 per cent for one to two hours in three groups of hydropenic subjects. These three groups consisted, respectively, of subjects without renal disease on normal salt intakes, similar subjects on salt-free diets treated with salt-retaining hormone, and patients with frank renal failure.

2. In each of the first two groups, comparable falls in filtration rate produced decreases in total urine osmolality averaging 10 per cent. In the majority of the subjects with renal failure, a fall in filtration rate produced a slight increase in urine osmolality.

3. In all three groups, the fall in filtration rate was associated with a marked drop in the rate of water and salt excretion, but with a lesser fall in the rate of potassium excretion.

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THE DISTRIBUTION OF EXTRACORPUSCULAR HEMOGLOBIN IN CIRCULATING PLASMA * †

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The addition of hemoglobin to plasma is accompanied by a number of physiochemical and biologic reactions. Among the important reactions which may occur is the interaction of hemoglobin with one or more of the plasma proteins in which hemoglobin forms a complex with, and becomes bound to, plasma protein. This phenomenon was first described by Polonovski and Jayle (*2*–4), and has been studied extensively (5, 6). To the protein (or proteins) having the property of binding hemoglobin Polonovski and Jayle gave the name haptoglobin (*7*). The identification of this protein(s) has not been definitely established, but tentative evidence suggests that it may be an α_2 globulin (5, 6). Utilizing *in vitro* and titra-metric techniques, Jayle and Conas (5) and others (6) have observed that plasma protein is capable of binding hemoglobin in concentrations up to approximately 150 mg. per cent; at concentrations in excess of this, additional binding does not occur. This limitation of binding capacity was later observed by Laurell and Nyman (8) and Allison and ap Rees (9) who used paper electrophoretic techniques and who also demonstrated that hemoglobin appeared in plasma in the free, unbound state when added *in vitro* in amounts which exceeded the maximal binding capacity.

The quantitative aspects of hemoglobin binding *in vitro* and of the relationships between binding capacity and the appearance of free hemoglobin have been established (8, 9). However, the methods available have not been suitable for *in vivo* studies and, consequently, quantitative information concerning these phenomena and the distribution of hemoglobin in *circulating* plasma is lacking. It was the purpose of the present study to provide such information. To this end an electrophoretic method was developed for the quantitative analysis of free and protein-bound hemoglobin concentrations in plasma. Using this method, a study was made of the electrophoretic characteristics of plasma hemoglobin and of the distribution and transport of intravenously administered hemoglobin.

METHODS

The total hemoglobin concentration in plasma was determined by the method of Evelyn and Malloy (10). The relative concentrations of free hemoglobin, protein-bound hemoglobin (PBH), and methemalbumin were determined by photometric analysis of benzidine-stained filter paper strips following separation of the individual hemeprotein fractions by paper electrophoresis.

Paper electrophoresis. Standard techniques were employed in which 10 μl. of plasma containing hemoglobin was subjected to overnight electrophoresis (5 ma., 110 volts) in a Durrum type cell employing hanging filter paper strips (Whatman No. 3). Two different buffer solutions were used: *1*) veronal buffer, pH 8.6, ionic strength, 0.075, and *2*) sodium phosphate buffer, pH 7.0, ionic strength, 0.05. For quantitative studies the phosphate buffer was routinely used.

Staining. The property of hemoglobin to catalyze the oxidation of benzidine into its colored, quinoid derivative in the presence of hydrogen peroxide was utilized for staining the separated hemoglobin fractions. On completion of electrophoresis the paper strips were placed in an oven at 120° C. for 30 minutes in order to fix the hemoglobin to the paper. After removal from the oven, the filter paper strips were individually immersed in a solution of 1 or *2* per cent benzidine in 20 per cent acetic acid which was prepared fresh each day. The strength of the benzidine employed varied with the hemoglobin concentration. At hemoglobin concentrations less than 200 mg. per cent, *2* per cent benzidine was used; at higher concentrations 1 per cent benzidine was employed. When thoroughly saturated with benzidine (immersion for 15 to 30 seconds) the paper strips were removed and were blotted thoroughly to remove excess benzidine. They were then immediately immersed, again individually, in a freshly prepared solution of 3 per cent hydrogen peroxide in water. The paper strips were thoroughly saturated

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with peroxide but were not immersed for longer than 30 seconds since progressive elution of oxidized benzidine from the paper occurred during immersion for longer periods. On removal from hydrogen peroxide the strips were rinsed quickly with distilled water to remove excess peroxide and were partially dried by blotting. The paper strips used for quantitative studies were set aside to allow the oxidation of benzidine to reach a maximum before photometric analysis was made. This required approximately five to seven minutes.

Quantitative analysis. When the oxidation of benzidine was complete and color intensity reached a maximum, measurements of the light absorption of the stained fractions were made. The damp filter paper strips were placed in a clear, celluloid envelope and were inserted in an automatic recording densitometer (Spinco Analytrol) for scanning. The celluloid envelope was used to prevent sticking or tearing of the damp filter paper during scanning. Using a 500 mμ interference filter, the light absorption of the stained paper strips was recorded automatically and measured. Using these measurements, the relative concentrations of free hemoglobin, PBH and methemalbumin were computed. The absolute concentrations of these fractions were calculated from the relative concentrations when these were related to the determined total hemoglobin concentration.

EXPERIMENTAL PROCEDURE

Hemoglobin was administered intravenously in either single or multiple injections to 13 healthy, fasting adult subjects who showed no evidence of cardiovascular, infectious, hematologic or renal disease. Hemoglobin was prepared from autogenous blood which was withdrawn in heparinized syringes and hemolyzed by the addition of 2.5 volumes of distilled water to each volume of whole blood. Using a blood transfusion set to prevent the introduction of clots, a total of approximately 14 to 16 Gm. of hemoglobin was administered as a 5 per cent solution. In 10 subjects this amount of hemoglobin was given over a two or three hour period in seven to nine evenly spaced injections, each injection containing approximately 2 Gm. of hemoglobin. In three subjects hemoglobin (14 to 16 Gm.) was administered in a single injection over a period of 30 minutes. Arterial blood samples were taken every 20 to 30 minutes following the initial injection and all subsequent injections, if given, over a period of from two to six hours. Blood was collected carefully in dry syringes utilizing the pressure of the arterial flow, care being taken to avoid hemolysis, and was placed in heparinized tubes, mixed gently and centrifuged. Following centrifugation the plasma was separated and was frozen and stored at −10° C. until analysis for hemoglobin content could be made.

OBSERVATIONS AND RESULTS

Electrophoretic characteristics

Plasma hemoglobin was separated electrophoretically into two components: free hemoglobin and protein-bound hemoglobin (PBH). The electrophoretic characteristics of these heme-proteins and their positional relationships to plasma proteins at pH 8.6 and at pH 7.0 are illustrated in Figures 1 and 2, respectively. At pH 8.6 free hemoglobin migrated between α_2 and β globulin and PBH migrated with, or slightly slower than, α_2 globulin. At pH 7.0 free hemoglobin did not migrate but remained at the site of application of the sample to the paper, assuming a position to the cathode side of the application site as a result of endosmotic flow. At this pH, PBH migrated with α_2 globulin. These electrophoretic characteristics are similar to those previously described (8, 9, 11, 12).

In addition to free and protein-bound hemoglobin, a third benzidine reacting material was separated electrophoretically. This fraction, tentatively identified as methemalbumin, migrated with albumin at pH 8.6 and 7.0 (Figures 1 and 2).

The identification of these fractions as free hemoglobin, PBH, and methemalbumin, respectively, was based upon the following considerations:

Free hemoglobin. *a*) The electrophoretic characteristics of this moiety were similar to those of preparations of hemoglobin in aqueous solution which were studied under similar conditions and are those to be expected of unbound hemoglobin with an isoelectric point of 6.9 (8); *b*) The glomerulus is permeable to this fraction which is excreted in the urine (13), indicating that it is diffusible and suggesting that it circulates in the free, unbound state.

Protein-bound hemoglobin. *a*) The electrophoretic characteristics of this moiety were similar to those of protein-bound hemoglobin as described by Smithies and Walker (11, 12), Laurell and Nyman (8) and Allison and ap Rees (9), and are those to be expected of hemoglobin which is believed to be bound by α_2 globulin (5); *b*) The demonstration, as described below, of a limiting, maximal concentration of this fraction, with the appearance of another moiety (free hemoglobin) at higher hemoglobin concentrations, is consistent with a protein-binding reaction as has been described for other constituents of plasma (14); *c*) This fraction is not excreted in the urine (13), an observation consistent with hemoglobin binding by a large-sized plasma protein to which the glomerulus is impermeable; *d*) The absorption spec-

trum of this fraction (Figure 3) was similar to that of oxyhemoglobin (15), suggesting that the benzidine reacting material which is bound to plasma protein is hemoglobin and not a derivative thereof.

Methemalbumin. The identification of this moiety, which migrated with albumin at pH 8.6 and 7.0, as methemalbumin was based upon its electrophoretic characteristics. These characteristics were similar to those of preparations of methemalbumin (acid hematin plus albumin) which were studied under similar electrophoretic conditions. Attempts at a more definitive identification by demonstration of the characteristic absorption spectrum of methemalbumin were unsuccessful, presumably because the concentrations involved were too small to detect spectroscopically.

A distinct electrophoretic separation was consistently obtained between free hemoglobin, PBH and methemalbumin at both pH 7.0 and 8.6. However, a greater separation was obtained between free and protein-bound hemoglobin at pH 7.0 owing to the stationary position of free hemoglobin at this pH. For this reason pH 7.0 was selected for routine quantitative analysis. Another factor in the selection of this pH for quantitative studies was that identification of the separated fractions was further simplified owing to the appearance of protein-bound hemoglobin as a single, easily recognizable broad band at pH 7.0, whereas at pH 8.6 PBH often appeared in double bands (Figure 1), the slowest moving of which was in close proximity to free hemoglobin. The appearance of PBH in multiple bands [up to four have been described using *in vitro* techniques and starch electrophoresis (9, 11, 12)] has been attributed to genetically determined differences of the protein(s) having the property of binding hemoglobin (11, 12). The merging of separate bands of PBH into a single homogenous fraction

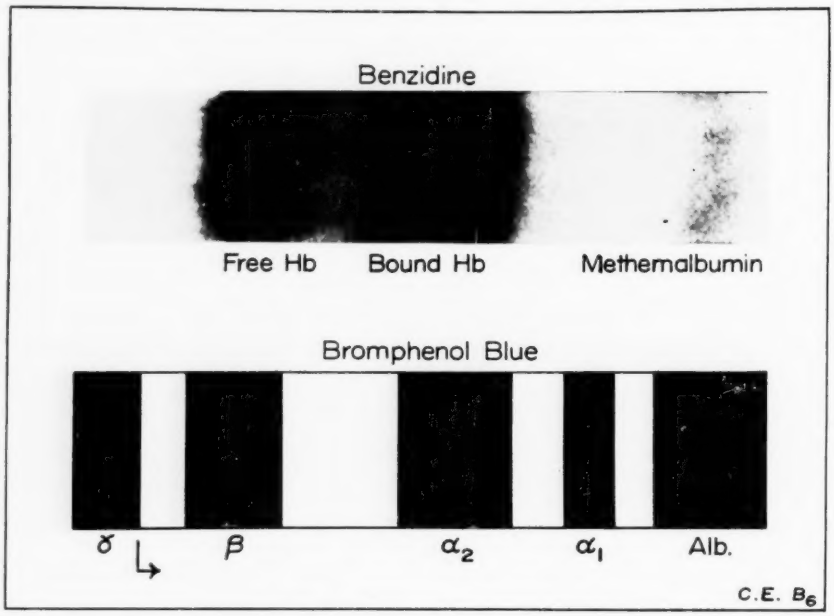

FIG. 1. THE ELECTROPHORETIC CHARACTERISTICS OF PLASMA HEMOGLOBIN AND PLASMA PROTEINS AT PH 8.6

The upper strip, stained with benzidine, shows the position of free hemoglobin, protein-bound hemoglobin (PBH), and methemalbumin. At this pH, PBH appeared as a double band. The lower strip shows schematically the positions of plasma proteins as determined by staining with bromphenol blue. The arrow refers to the application site and direction of movement towards the anode.

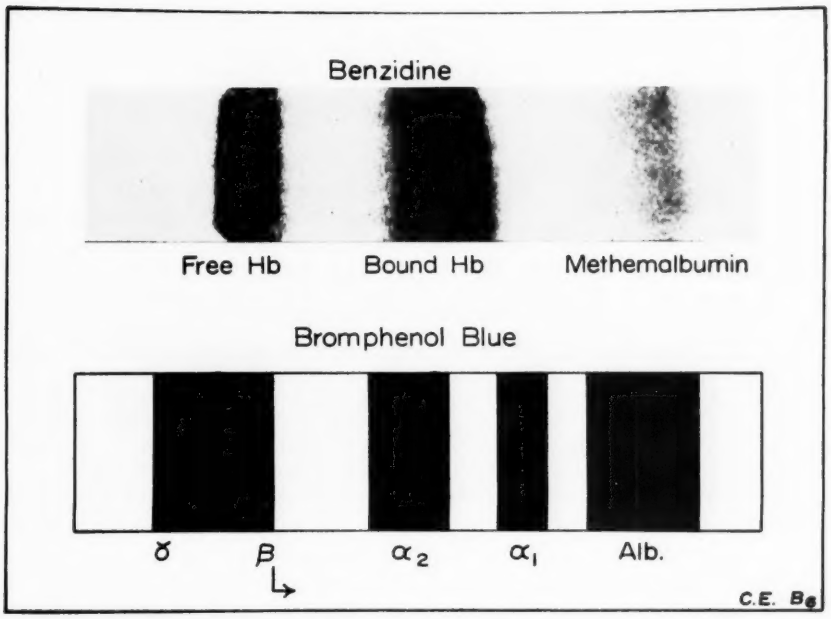

FIG. 2. THE ELECTROPHORETIC CHARACTERISTICS OF PLASMA HEMOGLOBIN AND PLASMA PROTEINS AT pH 7.0

At this pH, free hemoglobin assumed a position to the cathode side of the application site owing to endosmotic flow. PBH appeared as a single homogenous band.

at pH 7.0 may be attributed to a general reduction in electrophoretic resolution at this pH.

Staining characteristics

The blue color imparted to the filter paper strips appeared in the areas of the paper containing hemoglobin (or methemalbumin) within a few seconds after immersing the benzidine-saturated paper in hydrogen peroxide. The intensity of the color increased progressively with time after removal of the strips from peroxide, reaching a maximum in all fractions in from five to seven minutes, as determined photometrically. After this time interval the color remained stable for 10 to 15 minutes, during which time measurements of light absorption were made. After 15 to 30 minutes the background of the paper became discolored as a result of the decomposition of benzidine and photometric measurements were no longer possible.

The relative intensities of the three fractions did not change with variations in the exposure time to benzidine or hydrogen peroxide or with variable concentrations of these reagents as employed. The color intensity of each fraction was greater when 2 per cent benzidine was used as compared with 1 per cent, but the relative intensities of the three fractions did not change. Similarly, variability was encountered from day to day in the intensity of the color reaction obtained, but each fraction varied in a similar manner and the relative intensities remained unchanged. The factors accounting for this day to day variability were not established, but in view of the sensitive nature of the catalytic reaction involved in the oxidative transformation of benzidine, numerous factors may have contributed, such as minor variations in temperature, exposure time, saturation of the paper and concentration of the reagents employed.

During the development of color a slow diffusion of oxidized benzidine occurred through the wet filter paper. Such diffusion did not obscure the background between the separated fractions and had ceased at the time of photometric measurements when partial drying had taken place.

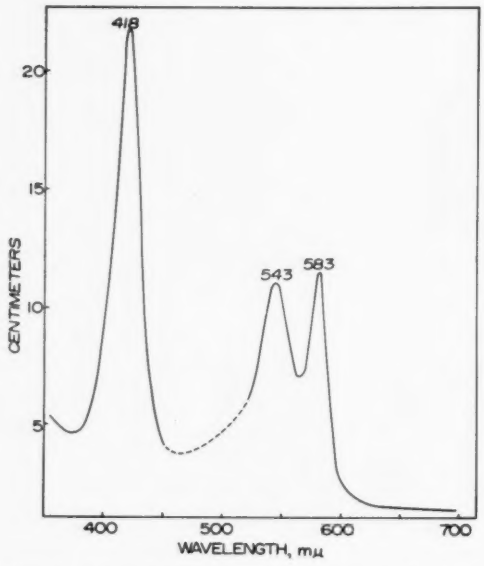

FIG. 3. ABSORPTION SPECTRUM OF PROTEIN-BOUND HEMOGLOBIN

The peaks at 543 and 583 mμ are characteristic of oxyhemoglobin. These peaks were determined at a plasma dilution of 1:1; the Soret band (418 mμ) was determined at a dilution of 1:7. The ordinate refers to the distance (cm.) of the excursion of the recording pen above the baseline, which was a function of extinction of light.

Quantitative analysis

The characteristic curves obtained when the light absorption of the benzidine stained paper strips was measured are illustrated in Figure 4. In order to establish the validity of the use of these curves for the determination of the relative concentrations of free hemoglobin, PBH and methemalbumin, studies were made of the relationship between hemoglobin concentration and the color intensity of the benzidine stained fractions. The purpose of these studies was twofold: *a*) to determine if the relationship between hemoglobin content and color intensity, as determined by light absorption, was linear, and *b*) to determine if the relationship between hemoglobin content and light absorption was the same for free hemoglobin, PBH and methemalbumin.

In order to study the first of these relationships, known concentrations of hemoglobin in aqueous solution were applied to filter paper, color was developed with benzidine and hydrogen peroxide in the usual manner, and photometric measurements were made. At concentrations of hemoglobin of 12.5 to 200 mg. per cent, the relationship between

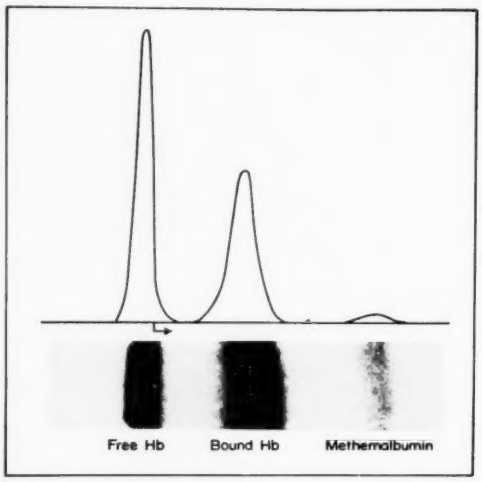

FIG. 4. CURVES OF THE LIGHT ABSORPTION OF BENZIDINE-STAINED HEMOGLOBIN FRACTIONS

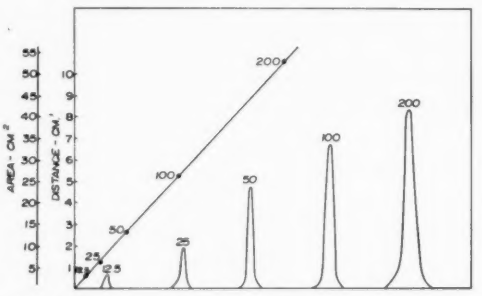

FIG. 5. RELATIONSHIP BETWEEN THE CONCENTRATION OF FREE HEMOGLOBIN AND LIGHT ABSORPTION FOLLOWING STAINING WITH BENZIDINE-HYDROGEN PEROXIDE.

Hemoglobin was applied to a filter paper strip in concentrations of 12.5 to 200 mg. per cent. The numbers within the enclosed box represent hemoglobin concentrations. The curves represent recorded measurements of the light absorption of the respective hemoglobin concentrations following staining with benzidine-hydrogen peroxide. The height of these curves is expressed as the distance (cm.) of the excursion of the recording pen above the baseline. The area under each curve is expressed as "area—cm.²" The diagonal line is a plot of the concentration of hemoglobin against the area under the respective curve of light absorption. The relationship shown between concentration and light absorption is linear.

concentration and light absorption was linear (Figure 5), indicating that the color intensity of the benzidine oxidatively transformed was directly proportional to the content of hemoglobin on paper.

In order to compare the relationship between hemoglobin concentration and light absorption for free and protein-bound hemoglobin, the following study was made. Hemoglobin was added to plasma *in vitro* in increments of from 2 to 5 mg. per cent over a concentration range of 80 to 240 mg. per cent. Following incubation of plasma at 37° C. in order to promote protein-binding, electrophoresis, staining and photometric analysis were carried out in the usual manner. Protein-binding was maximal in this experiment at a hemoglobin concentration of 112 mg. per cent. At concentrations greater than 112 mg. per cent, free hemoglobin appeared in increasing concentrations. Assuming that the concentration of PBH remained at 112 mg. per cent at all concentrations in excess of this maximal binding capacity, the concentration of free hemoglobin at these higher (112 to 240 mg. per cent) concentrations was calculated as the difference between the known *total* hemoglobin concentration and the concentration of PBH (112 mg. per cent). Knowing, therefore, the actual concentrations of free and protein-bound hemoglobin, the light absorption of each fraction was measured and compared at each concentration studied. Figure 6 illustrates that at all concentrations studied the relationship between hemoglobin concentration and light absorption of the oxidized benzidine bands was linear and was the same for free and protein-bound hemoglobin. Calculations of the concentrations of free and protein-bound hemoglobin as determined by the photometric technique were, therefore, in close agreement with the actual concentrations of these components (Table I).

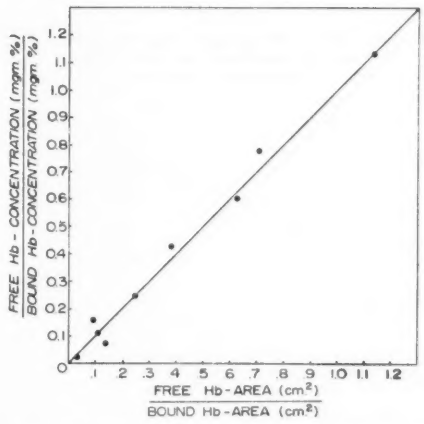

FIG. 6. RELATIONSHIP BETWEEN CONCENTRATION AND LIGHT ABSORPTION (BENZIDINE STAIN) OF FREE HEMOGLOBIN RELATIVE TO PROTEIN-BOUND HEMOGLOBIN

The concentration of free hemoglobin relative to the concentration of protein-bound hemoglobin is plotted against the respective ratios of the light absorption of these constituents (when stained with benzidine) at each ratio of concentrations studied. The straight line relationship shown indicates that the relationship between concentration and light absorption is the same for free and protein-bound hemoglobin.

A similar study of methemalbumin revealed that the relationship between concentration and

TABLE I

In vitro study of the recovery of free and protein-bound hemoglobin (Hb) as determined by photometric analysis *

Total Hb	Free Hb		Free (calculated) / Free (actual)	Bound Hb		Bound (calculated) / Bound (actual)
	Actual	Calculated		Actual	Calculated	
110	0	0	1.00	110	110	1.00
112	0	0	1.00	112	112	1.00
115	3	3	1.00	112	112	1.00
120	8	14	1.75	112	106	0.95
125	13	12	0.93	112	113	1.01
130	18	12	0.67	112	118	1.05
140	28	30	1.07	112	110	0.98
160	48	44	0.92	112	116	1.04
180	68	69	1.01	112	111	0.99
200	88	90	1.02	112	110	0.98
240	128	128	1.00	112	112	1.00

* Hemoglobin values are expressed as concentration in mg. per cent.

TABLE II

In vitro study of the recovery of free hemoglobin (Hb) and methemalbumin as determined by photometric analysis *

Free Hb		Methemalbumin	
Actual	Calculated	Actual	Calculated
5	7	5	3
10	11	10	9
20	20	20	20
30	28	30	32
40	41	40	39

* All values are expressed in mg. per cent.

light absorption was the same for this heme-protein as for free hemoglobin (and, by inference, therefore, the same as PBH). In this experiment methemalbumin, prepared by adding acid hematin to albumin, was mixed with equal concentrations of free hemoglobin in aqueous solution over a range of concentration of each of from 5 to 40 mg. per cent. The similar relationships between light absorption and concentration obtained for free hemoglobin and methemalbumin resulted in a close agreement found between the actual and calculated concentrations of these constituents when light absorption measurements were used to estimate concentrations (Table II).

These experiments therefore satisfactorily established the validity of the quantitative photometric method as employed. The necessary criteria for validation were fulfilled: The relationship between concentration and light absorption was linear and was the same for free hemoglobin, PBH and methemalbumin.

The sensitivity of this quantitative method was such that hemoglobin concentration was accurately measured within ± 5 mg. per cent (Table I). At concentrations in excess of 20 mg. per cent the experimental error of this method, as determined by multiple analysis of single samples of plasma, was ± 7.0 per cent. These figures apply to calculations of free hemoglobin, PBH and methemalbumin.

Application of quantitative method—The distribution of intravenously administered hemoglobin

The distribution of hemoglobin in plasma following intravenous administration is illustrated by representative experiments in Figures 7 and 8. These figures illustrate, respectively, the distribution of hemoglobin when the plasma level was

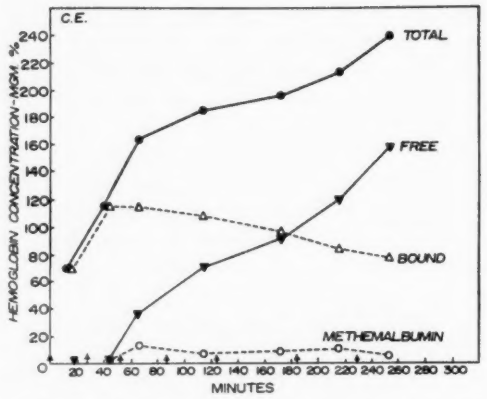

FIG. 7. THE DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED HEMOGLOBIN

At each point in time indicated by an arrow approximately 2 Gm. of hemoglobin was given intravenously. With time the total hemoglobin concentration progressively increased. Initially all hemoglobin circulating in plasma was bound to plasma protein. When the maximal binding capacity was exceeded (114 mg. per cent) free hemoglobin appeared in plasma, the concentration of which progressively rose. Accompanying the appearance of free hemoglobin, a small quantity of methemalbumin was formed. With time, the concentration of PBH fell progressively.

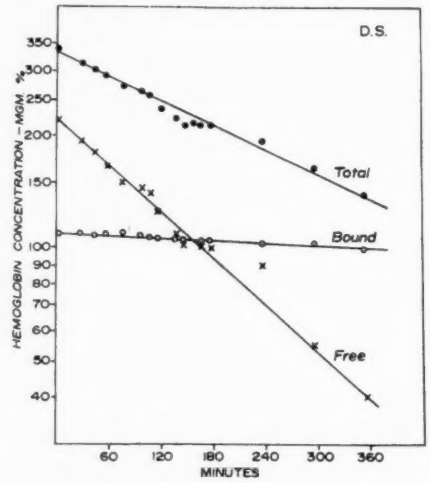

FIG. 8. THE DISTRIBUTION OF HEMOGLOBIN IN PLASMA FOLLOWING A SINGLE LARGE INTRAVENOUS INJECTION

Both free and protein-bound hemoglobin were present initially. The concentration of each decreased with time. The disappearance of both free and protein-bound hemoglobin was exponential, but free hemoglobin disappeared at a faster rate.

gradually increased, and the disappearance of hemoglobin from plasma following rapid elevation of the plasma level.

When hemoglobin was introduced into circulating blood it was immediately and, insofar as could be determined, instantaneously bound to plasma protein (Figure 7). When small quantities were administered, binding was quantitative and hemoglobin circulated entirely in the protein-bound state. As hemoglobin was given in increasing amounts, the quantity bound increased until a concentration was attained at which additional hemoglobin given was not bound but instead circulated in the free, unbound state (Figure 7). This limiting concentration, which may be considered as the maximal binding capacity of the hemoglobin-binding protein, varied in individual studies from 98 to 171 mg. per cent (Table III) and averaged 128 ± 25 mg. per cent. These capacities are similar to those observed *in vitro* by Laurell and Nyman (8) and Allison and ap Rees (9).

TABLE III

In vivo distribution of hemoglobin (Hb) in plasma *

Subject		Total Hb mg. %	PBH mg. %	Free Hb mg. %	Methemalbumin mg. %
V. T.	1)	171	171	0	0
	2)	355	145	210	0
R. R.	1)	162	162	0	0
	2)	258	142	101	5
J. W.	1)	143	143	0	0
	2)	325	53	263	9
B. K.	1)	133	133	0	0
	2)	230	68	157	5
P. R.	1)	133	133	0	0
	2)	265	74	182	9
C. E.	1)	114	114	0	0
	2)	239	77	157	5
I. R.	1)	111	111	0	0
	2)	269	60	200	9
E. D.	1)	107	107	0	0
	2)	320	79	241	0
L. S.	1)	99	99	0	0
	2)	205	43	151	11
L. C. S.	1)	98	98	0	0
	2)	280	43	228	9

* As determined under conditions of a progressively increasing hemoglobin concentration. 1) Refers to the hemoglobin concentrations at the maximal binding capacity of plasma protein for hemoglobin. 2) Refers to the final concentrations of hemoglobin at the completion of the experiment.

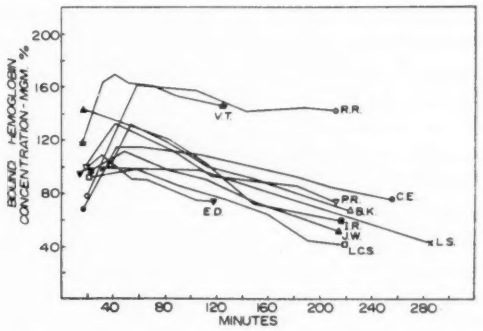

FIG. 9. DISAPPEARANCE CURVES OF PROTEIN-BOUND HEMOGLOBIN FROM PLASMA

These curves, obtained from 10 separate experiments under conditions of a progressively increasing *total* plasma hemoglobin concentration, illustrate the initial rise in the concentration of PBH to a maximal level with subsequent progressive fall in concentration with time.

The limitation imposed by this maximal binding capacity resulted in hemoglobin appearing in plasma in the free, unbound state when the amount of hemoglobin administered was in excess of that which could be bound. Under these circumstances, a progressive increase occurred in the concentration of free hemoglobin circulating in plasma as additional hemoglobin was administered intravenously. No limitation was imposed upon the transport of hemoglobin in the unbound state. Concentrations up to 263 mg. per cent were obtained (Table III).

Accompanying this rise in total and free hemoglobin levels in plasma, the concentration of PBH diminished with time (Figure 9). The rate at which this decrease in concentration of PBH occurred varied in individual studies from 6 to 25 mg. per cent per hour and averaged 15 mg. per cent per hour. In five subjects (E.D., P.R., B.K., I.R. and V.T.) PBH disappeared from plasma at a single exponential rate ($T_{1/2}$, 250 minutes). In the remaining five subjects the disappearance was linear and not exponential. The disappearance rate, however, was approximately the same in these subjects as in those in whom an exponential disappearance occurred. The disappearance of PBH from plasma has been studied by Laurell and Nyman (8) and was observed by them to be accompanied by a parallel decrement in the concentration

of α_2 globulin, suggesting that hemoglobin is removed from the circulation in combination with the protein to which it is bound. Since PBH is not excreted in the urine (13) this removal must occur at extravascular sites within the body, presumably within the reticuloendothelial system (16). The capacity of this or other systems to remove PBH has not been established, but Laurell and Nyman have suggested that this capacity may be of a low order of magnitude in view of a constant rather than an exponential disappearance rate as observed by them in two subjects (8). The present observations do not clarify this supposition since the characteristics of the disappearance curves were not consistent. The explanation of this inconsistency is not clear. Conceivably, differences in disappearance rates might occur as a result of differences among individual subjects in the characteristics of the hemoglobin-binding protein which have been described (11, 12). However, the differences in disappearance curves observed may be artificial owing to an inadequate time of observation. In view of the slow disappearance rates, a longer period of observation is necessary to establish more clearly the precise characteristics of the removal of PBH from plasma.

Accompanying these changes in plasma hemoglobin, methemalbuminemia was observed in 8 of 10 studies (Table III). The appearance of methemalbuminemia was dependent upon the presence of free hemoglobin in plasma. Methemalbumin was not formed until the binding capacity was exceeded and hemoglobin appeared in the free, unbound state (Figure 7). This observation, also made *in vitro* by Allison and ap Rees (9), is consonant with the view (9, 17) that methemalbumin is formed *in vivo* from heme molecules which are derived from free but not from protein-bound hemoglobin.[1] There was, however, no correlation observed in the present experiments between the plasma level of methemalbumin and the concentration of free hemoglobin. When methemalbuminemia developed, it did so early, when free hemoglobin first appeared, or not at all (Figure 7). The quantity of methemalbumin formed was small. The plasma levels varied in individual studies from 5 to 11 mg. per cent (Table III). These levels remained relatively constant during the period of observation and neither decreased nor increased significantly as the level of free hemoglobin changed. This suggests that methemalbumin was probably formed and removed from the circulation at a relatively constant rate.

In Figure 8 a representative experiment is illustrated of the disappearance from plasma of free and protein-bound hemoglobin following rapid elevation of the plasma levels of these constituents by a single, rapid, intravenous injection of hemoglobin. In this and two other similar experiments the initial total hemoglobin concentration was greater than 200 mg. per cent and both free and protein-bound hemoglobin were present in the initial samples. Methemalbuminemia did not occur in these three studies. With time the concentrations of free and protein-bound hemoglobin decreased. At the completion of the period of observation (five to six hours) the concentration of PBH had fallen by 27, 18 and 32 per cent, respectively, in the three studies and free hemoglobin by 62, 82 and 59 per cent. Free hemoglobin disappeared from plasma at an exponential rate (average $T_{1/2}$, 207 minutes). The characteristics of the disappearance curves of PBH were variable. In Subject D.S. (Figure 8), PBH disappeared at a slow exponential rate. In the other two subjects, the disappearance was constant and was not exponential. These differences, as noted above, remain unexplained. The faster disappearance of free hemoglobin (Figure 8) than of PBH may be attributed, in part, to its excretion in the urine (13) and to its smaller molecular size with consequent larger volume of distribution in body fluids. Whether free and protein-bound hemoglobin are removed differentially by the reticuloendothelial or other systems remains to be determined.

[1] Small quantities of methemalbumin were detected in plasma containing only PBH after storage in the frozen state for longer than two to three months. Thus, heme molecules appear to be released from PBH *in vitro*, but not *in vivo*.

SUMMARY

Utilizing paper electrophoretic techniques, a method was developed for the partition and quantification of plasma hemoglobin. Electrophoretically, plasma hemoglobin was separated into a free and a protein-bound component. These fractions were identified, when benzidine and hydrogen

peroxide were used for staining purposes, by their characteristic positional relationships on paper and were quantified by photometric analysis of the stained filter paper strips.

Using these techniques a quantitative study was made in human subjects of the distribution and transport of intravenously administered hemoglobin. The observations made showed that hemoglobin was transported in plasma in a protein-bound form (PBH) and in a free, unbound state; that the binding of hemoglobin by plasma proteins was limited to a maximum hemoglobin concentration which averaged 128 ± 25 mg. per cent; that free hemoglobin did not appear in plasma until this binding capacity was exceeded; and that methemalbuminemia often developed, but only in association with the appearance of free hemoglobinemia.

From an analysis of rising and falling plasma hemoglobin levels, PBH was found to disappear slowly from circulating plasma and was distributed in extravascular sites, but was not excreted. Free hemoglobin disappeared from plasma at a faster rate than PBH and was excreted in the urine.

These observations emphasize the differential transport and distribution of free and protein-bound hemoglobin in circulating plasma. The methods employed provided quantitative information concerning these phenomena and may be used for further study of hemoglobin metabolism and physiology.

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EFFECT OF GROWTH HORMONE ON PLASMA FATTY ACIDS *

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Administration of growth hormone to animals has been found to reduce fat stores (1, 2), to increase liver fat (3, 4) and to cause ketosis (5) and a depression of respiratory quotient (6, 7). The increase in mobilization and metabolism of fat implied by these findings has been thought to contribute either in an auxiliary or in an essential way to the anabolic stimulus induced by growth hormone.

The recent work of Dole (8) and of Gordon and Cherkes (9, 10), indicating a relationship between the concentration of plasma unesterified fatty acids and adipokinesis, suggested another approach to the study of the effects of growth hormone on fat mobilization and offered a means of studying the effect in man.

METHODS AND MATERIALS

Plasma fatty acids were extracted and titrated according to the method of Dole (8). The term "fatty acids" as used in this paper is synonymous with nonesterified acids (NEFA) and unesterified fatty acids (UFA).

Purified preparations of human, simian and porcine growth hormone were made by the glacial acetic acid extraction method (11, 12). "Partially purified porcine growth hormone (acetic fraction)" refers to material extracted in the same way and treated with oxidized cellulose to remove corticotropin and intermedin, but not carried through the final precipitations.

Human and porcine corticotropin were prepared by the oxycel method of Astwood, Raben, Payne and Grady (13).

Purified bovine growth hormone (NIH-BGH-1) and ovine lactogenic hormone (NIH-SP-1) were gifts from the Endocrinology Study Section, National Institutes of Health. These and Thyrotropar® (bovine thyrotropin) were prepared by Armour and Company.

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RESULTS

Studies in dogs

Plasma fatty acid values were elevated by fasting and depressed by the administration of food, glucose, or insulin, as previously noted by others (9, 10). In both normal and diabetic dogs the morning fasting value was increased 100 per cent or more in each dog after an injection of 80 to 100 mg. of partially purified porcine growth hormone (acetic fraction) administered the preceding afternoon. The results of 22 determinations in three normal dogs and 15 determinations in three diabetic dogs are shown in Figure 1.

Although growth hormone raised the fasting value of fatty acids, it did not prevent the fall that occurred with infusions of glucose or glucose and insulin. Despite the administration of 80 mg. of acetic fraction both 18 hours before and again at the start of an intravenous infusion, injection of glucose at a rate of 1 Gm. per Kg. per hour into

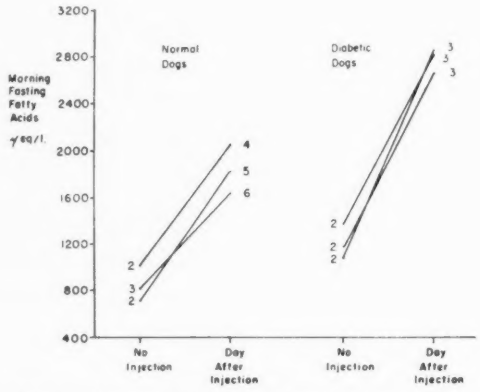

FIG. 1. FASTING VALUES OF PLASMA FATTY ACIDS IN NORMAL AND DIABETIC DOGS WITH AND WITHOUT GROWTH HORMONE

Eighty to 100 mg. of acetic fraction was administered 18 hours earlier. Each point is the average of the indicated number of determinations.

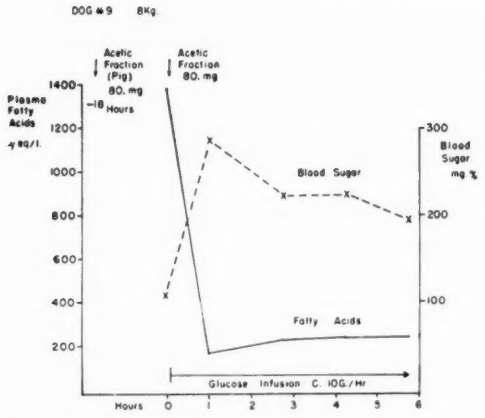

FIG. 2. THE EFFECT OF GLUCOSE ON FATTY ACID VALUES IN A DOG PRETREATED WITH GROWTH HORMONE

a dog anesthetized with sodium pentobarbital produced a fall in fatty acids from 1,400 to 180 μEq. per L. in one hour and the value remained low during the six hour infusion (Figure 2). A fall in concentration of variable magnitude was observed without glucose in dogs anesthetized with pentobarbital, but the depressant effect of glucose was much greater than that obtained with anesthesia alone.

In a similar experiment, but with insulin as well as glucose in the infusion medium, the value again fell despite prior growth hormone administration, but large doses of epinephrine, 0.2 mg. and 0.25 mg. given 10 minutes apart intravenously, caused a rapid rise in fatty acids despite the continued infusion. Fatty acids rose from 250 μEq. per L. to 1,220 μEq. per L. 30 minutes after the first injection. Growth hormone differed from epinephrine, which had previously been shown to elevate fatty acids (8, 9), in producing a slower response and a more sustained rise after a single injection and in the complete suppression of its effect by glucose.

Alloxan diabetic dogs were more sensitive than normal dogs to the effect of growth hormone. The difference in sensitivity was more apparent when sampling was performed seven hours after the daily meal rather than after a 24 hour fast. Growth hormone administered four hours before the determination greatly increased the values in diabetic dogs but affected normal dogs only slightly. In this experiment growth hormone elevated the values before a spontaneous postabsorptive rise occurred, suggesting that food suppresses the growth hormone effect less effectively in the diabetic dog.

The diabetic dogs responded to the smallest doses of purified growth hormone preparations used, 0.03 mg. per Kg. Fatty acid values in dogs were increased with purified preparations of porcine, bovine, human and simian growth hormone in contrast to the values in man which were affected only by the human and simian materials. The responsiveness of dogs to human growth hormone appeared to diminish over some weeks with repeated injections, whereas human subjects showed no loss in sensitivity over much longer periods.

Studies in man

The fasting value of plasma fatty acids in man was increased within four hours by the injection of human growth hormone. Fasting samples were obtained between 8:00 and 10:00 a.m.; the test substance was then administered intramuscularly and another blood sample was obtained four hours after the injection.

In the first group of 11 tests in five subjects, the average plasma fatty acid value rose from 555 μEq. per L. to 1,107 μEq. per L. following administration of 3 to 8 mg. of human growth hormone. In an equal number of trials without hormone, the average values were 642 μEq. per L. initially and 628 μEq. per L. four hours later. The individual

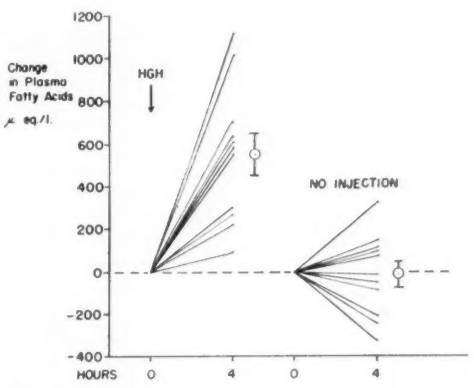

FIG. 3. EFFECT OF 3 TO 8 MG. OF HUMAN GROWTH HORMONE ON FASTING PLASMA FATTY ACID VALUES IN HUMAN SUBJECTS

responses are shown in Figure 3. The time of maximal effect was not established but was probably longer than four hours. Four mg. of human growth hormone raised the value for at least 24 hours in hypopituitary subjects, but usually for less than this period in normal subjects.

Three subjects, two normal adult males and an elderly hypopituitary male, were tested repeatedly over a six month period. The four hour change in fatty acids exceeded 400 μEq. per L. in 16 or 18 trials with hormone and in none of 14 trials without hormone (Figure 4). The minimal effective dose in the normal subjects was approximately 1.0 and 2.0 mg., while the hypopituitary subject had a substantial response from 0.5 mg., the smallest dose used. Other hypopituitary subjects were also found to be more sensitive than normal individuals, and some normal subjects required larger doses for a definite effect than the subjects of Figure 4.

As in the dog, glucose and food abolished the fatty acid response to growth hormone. Figure 5 illustrates a study in a 73 year old hypopituitary male in whom two control (2:00 p.m.) fasting values were 427 and 366 μEq. per L. on two different days. Injection of 4.0 mg. of human growth hormone at 10:00 a.m. produced a 2:00 p.m. value of 1,457 μEq. per L. When breakfast was allowed and 125 Gm. of glucose was ingested (50 Gm. at 9:30 a.m.; 50 Gm. at 12:15 p.m.; 25 Gm. at 1:30 p.m.), the 2:00 p.m. value was lowered to 130 μEq. per L. despite the injection of the same amount of growth hormone.

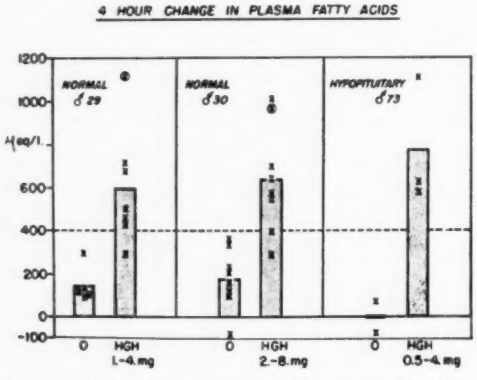

FIG. 4. CHANGE IN FATTY ACID VALUES IN TWO NORMAL AND ONE HYPOPITUITARY SUBJECT TESTED REPEATEDLY OVER SIX MONTHS

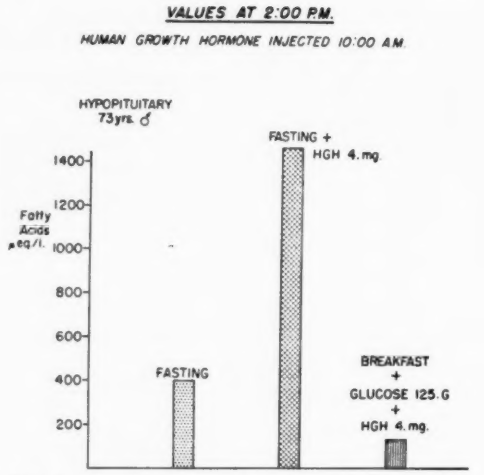

FIG. 5. INHIBITION OF THE PLASMA FATTY ACID RESPONSE TO HUMAN GROWTH HORMONE WITH FOOD AND GLUCOSE

Simian growth hormone was also active in man but porcine and bovine preparations were inert. All values four hours after 3 to 8 mg. of human growth hormone and after 4 to 20 mg. of simian growth hormone exceeded 1,000 μEq. per L. and all values on control days or after other pituitary preparations were below this figure (Figure 6). These included values after 20 to 30 mg. of porcine and 30 mg. of bovine growth hormone, 10 units of Thyrotropar®, 25 mg. of lactogenic hormone, and 50 to 400 clinical units of porcine and human corticotropin.

Larger effects might have been expected with corticotropin which was shown by Rosenberg (14) to increase liver fat in the mouse, and by White

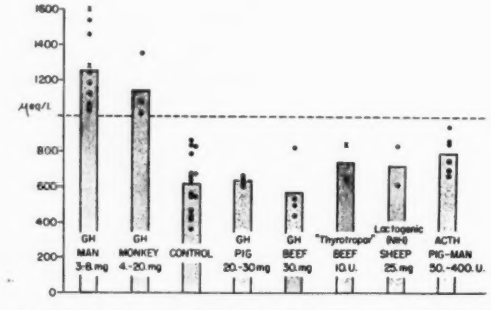

FIG. 6. PLASMA FATTY ACID VALUES IN THE THREE SUBJECTS OF FIGURE 4 FOUR HOURS AFTER TEST SUBSTANCES AND ON CONTROL DAYS

and Engel (15) and by Astwood (16) to stimulate fatty acid release from rat adipose tissue when added *in vitro*. The small response to corticotropin was apparently not due to adrenal stimulation, since cortisone neither lowered the fatty acid value nor inhibited the response to growth hormone.

In the subjects tested repeatedly over a six month period no loss in responsiveness to human growth hormone was observed and the material was invariably well tolerated. Most subjects noted no subjective effects, but three reported an increased libido and an obese woman on a 400 calorie diet reported a diminution in appetite when receiving human growth hormone (5.0 mg. per day). Blood sugar values were not affected in these tests.

DISCUSSION

The small doses of human growth hormone needed to elevate plasma fatty acids suggest a physiological role for this hormone in fat mobilization as well as in growth. Indeed, if corrected for body weight, the dose necessary for fatty acid elevation may be smaller than that required for growth, although little information is as yet available as to the amount needed for growth in man (17).

The suppression of the adipokinetic effect by food provides a mechanism of control not requiring changes in growth hormone secretion. Growth hormone could control, at least in part, the rate of fat mobilization in the postabsorptive state and yet be present at all times to direct the available energy whether from food or fat depots, towards protein synthesis and protein sparing. A pattern such as this would make unnecessary rapid fluctuation in hormone level in response to rapidly changing conditions. The long survival of injected growth hormone (18) makes it indeed unlikely that rapid changes in available hormone can occur.

The decrease in fatty acids after glucose in a hypopituitary subject, as also noted in this study, suggested to Dole, Bierman and Roberts (19) that the control of the release of fatty acids was independent of the pituitary. It is now seen that growth hormone accelerates fat mobilization beyond the rate induced by fasting in the absence of the pituitary.

Human growth hormone prepared by the acetic acid extraction method is virtually free of other known anterior and posterior lobe hormones, and thus its adipokinetic effect must be due to growth hormone or to a yet unidentified factor present in the preparation. The specificity of the human response to human and simian preparations noted in this study is compatible with other evidence of growth hormone behavior in man. Porcine and bovine preparations have appeared active only occasionally in many metabolic balance and growth studies. Human preparations have been regularly active and simian hormone, though less extensively tested, has appeared to be active in metabolic balance studies (20–24). The fatty acid response may indeed provide a rapid test for the effectiveness of growth hormone preparations in man (25).

SUMMARY

Fasting values of plasma unesterified fatty acids were raised in man by human and simian growth hormone and in the dog by human, simian, porcine and bovine growth hormone. Glucose, glucose and insulin, and food suppressed the effect of growth hormone on fatty acids. It was inferred that growth hormone influences the rate of fat mobilization in the postabsorptive period.

ACKNOWLEDGMENT

We are grateful to Miss Mary E. Pasciuto for technical assistance in this study.

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CORRECTION

In the paper by Scheinberg and Morell entitled "Exchange of Ceruloplasmin Copper with Ionic Cu^{64} with Reference to Wilson's Disease," in volume 36, pages 1193–1201, line 3 of page 1193 should read ". . . 15 to 30 mg. per 100 ml." In addition, columns 6 and 8 of Table IV on page 1197 are in error. The values given should read as follows:

.....Ceruloplasmin $\frac{\Delta O.D._{810}^{hem}}{\div 0.34}$	.....Enzymatic activity
mg./ml.	ΔO.D./min.
0.548	0.0546
0.380	0.0459
0.494	0.0399
0.576	0.0510
0.424	0.0408
0.448	0.0393

THE PHOSPHOLIPID COMPOSITION OF HUMAN SERUM LIPOPROTEIN FRACTIONS SEPARATED BY ULTRACENTRIFUGATION *

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(Submitted for publication August 19, 1958; accepted November 13, 1958)

Since essentially all of the lipid of human serum appears to be present in the form of lipoprotein complexes which can be separated into distinct fractions by electrophoresis, chemical fractionation, or ultracentrifugation techniques, the composition of these lipoprotein fractions has attracted interest. Consequently, lipoprotein fractions have been analyzed for protein, cholesterol, cholesterol ester, triglyceride and total phospholipid content. The phospholipid of serum, however, is composed of several components which may have different functional roles. Since in previous studies (1, 2) it was shown that the principal serum phospholipid components, consisting of lecithin, sphingomyelin, lysolecithin, and phosphatidyl ethanolamine, could be separated by chromatography on silicic acid and then quantitated, the present investigation was carried out to determine the distribution of the individual phospholipids among different lipoprotein fractions. To isolate the lipoprotein fractions, the technique of ultracentrifugation at various serum densities (3, 4) as modified by Havel, Eder and Bragdon (5) was used. Lipoprotein fractions of density < 1.019, < 1.063 and > 1.063 were then analyzed for the individual phospholipids, cholesterol, cholesterol ester and triglyceride.

MATERIALS AND METHODS

Serum samples were obtained in the fasting state from eight apparently healthy white adults (Table I).

Ultracentrifugation of the serum, begun within two to three hours after withdrawal, was accomplished according to the method of Havel, Eder and Bragdon (5). The samples were centrifuged at $105,000 \times G$ at 15° C. in the No. 40 rotor of a Model L Spinco ultracentrifuge. Each serum sample (except that from Subject E.Ri.) was divided into three parts and processed as follows: *1*) Five to 8 ml. of serum was diluted to a density of 1.019 using a NaCl-KBr solution of density 1.085, centrifuged for 22 hours, and the top layer removed and extracted; *2*) Four to 6 ml. of serum was diluted to a density of 1.063 using a NaCl-KBr solution of density 1.346, centrifuged for 22 hours, and the top and bottom layers separated and extracted; *3*) Three to 5 ml. of untreated serum was extracted. In all of the samples, centrifugation resulted in a distinct layer at the top and bottom of the tube with a colorless zone about one-half the length of the tube in between. The upper layer of the 1.019 density tube was usually milky. The fractions were separated by slicing the tube with a Spinco tube slicer at approximately the center of the colorless zone.

The serum samples, which were not dialyzed before extraction, were extracted with methanol-chloroform (1:1, v/v) and the extracts chromatographed on silicic acid columns (1 Gm.) in order to determine the contents of the individual phospholipids, cholesterol, cholesterol ester and triglyceride (2). Certain of the extracts were also chromatographed on silicic acid-impregnated filter paper (Whatman No. 3) using 20 per cent methanol in chloroform (v/v) as solvent. In addition, column fractions were hydrolyzed in 6 N HCl at 110° C. for 18 hours in a sealed tube and chromatographed on filter paper (Whatman No. 1) using butanol-acetic acid-water (4:1:4, using butanol layer) as solvent for the nitrogenous components and acetone-water (4:1) as solvent for inositol. These paper chromatograms were developed by *1*) spraying with ninhydrin in butanol, followed by heating at about 110° C. for five minutes, *2*) the phosphomolybdic acid stain of Chargaff, Levine and Green (6), and *3*) the stain for inositol of Trevelyan (7) as modified by Anet and Reynolds (8).

The column fractions were analyzed for phosphorus by the method of Fiske and Subbarow (9), for cholesterol and cholesterol ester by the method of Schoenheimer and Sperry (10) as modified by Sperry and Webb (11), and for ester bonds by the method of Rapport and Alonzo (12).

RESULTS

The distribution of the total serum phospholipid among the three density fractions (< 1.019, < 1.063, > 1.063) of lipoproteins is recorded in Table I. The fraction with density < 1.063 includes the fraction with density < 1.019, and the values for a fraction with density 1.019 to 1.063 can be calculated by difference. Although there was considerable variation between subjects in the

* This investigation was supported in part by a research grant (H-2907) from the National Heart Institute, Public Health Service, and in part by a gift from the American Heart Association.

TABLE I

Distribution of serum phospholipids in density fractions

Subject	Sex	Age	Density of fraction <1.019		<1.063		>1.063		Recovery*	Total serum lipid P
			mM/L.	*% total*	*mM/L.*	*% total*	*mM/L.*	*% total*	*%*	*mM/L.*
E. R.	M	22	0.94	34.0	1.47	54	1.28	46	95	2.90
N. R.	M	23	0.45	20.0	0.91	40	1.36	60	88	2.58
H. D.	M	24	0.21	8.3	1.06	43	1.43	57	92	2.70
E. Ri.	M	30			2.02	52	1.86	48	91	4.27
A. G.	M	39	0.76	26.0	1.35	46	1.59	54	96	3.06
D. G.	F	26	0.31	11.0	1.09	38	1.77	62	100	2.86
A. R.	F	31	0.28	8.4	1.42	43	1.88	57	100	3.30
R. D.	F	31			1.62	45	1.99	55	90	4.01
Average			0.49	18.0	1.37	45	1.65	55	94	3.21

* Per cent recovery of total serum lipid P from 1.063 density tube after centrifugation.

percentage of total serum phospholipid in the fraction with density < 1.019, the fraction with density < 1.063 showed less individual variation. The mean phospholipid content was somewhat greater in the fraction with density > 1.063 than in the fraction with density < 1.063. The average recovery of phospholipid in the totaled separated fractions as compared to whole serum was 94 per cent.

The concentrations and relative distributions of the individual phospholipids of each density fraction are listed in Tables II through IV. The average relative distribution of the individual phospholipids of whole serum in these subjects was as follows: "cephalin," 4.8 per cent; lecithin, 68.2 per cent; sphingomyelin, 18.9 per cent; and lysolecithin, 8.0 per cent. The "cephalin," although a mixture, is composed predominantly of ethanolamine-containing phospholipid (2). As can be seen in these tables, all four of the individual phospholipids were found in each density fraction. Moreover, the relative molar proportions of the individual phospholipids in the fraction with density < 1.019 and in the fraction with density < 1.063 were quite similar. But when comparing the values in these fractions to those of the fraction with density > 1.063, striking differences were apparent. Although the relative amounts of "cephalin" and of lecithin were similar, there was relatively less sphingomyelin and more lysolecithin in the fraction with density > 1.063. In fact, even though the fraction with density > 1.063 included on an average a little more than half the total serum phospholipid, it contained about 40 per cent of the sphingomyelin and 75 per cent of the lysolecithin. To illustrate this point, the molar ratios of sphingomyelin-to-lecithin and of "cephalin"-to-sphingomyelin in the fractions with density < 1.063 and density > 1.063 are tabulated in Table V. From these figures, it is apparent that in the fraction with density < 1.063, the mean molar sphingomyelin-to-lecithin ratio was twice, and the mean molar "cephalin"-to-sphingomyelin ratio about one-half, the value found in the frac-

TABLE II

Phospholipid composition of fraction with density <1.019 *

Subject	"Cephalin"		Lecithin		Sphingomyelin		Lysolecithin	
	mM/L.	*% total*†	*mM/L.*	*% total*†	*mM/L.*	*% total*†	*mM/L.*	*% total*†
E. R.	0.043	4.6	0.59	62.6	0.26	27.2	0.053	5.6
N. R.	0.030	6.6	0.31	68.8	0.09	19.9	0.021	4.7
A. G.	0.038	5.0	0.50	66.1	0.18	23.8	0.039	5.1
A. R.	0.018	6.4	0.19	67.4	0.06	21.4	0.014	5.0
Average	0.032	5.7	0.40	66.2	0.15	23.1	0.032	5.1

* Samples on other subjects either not done or too inaccurate because of small quantity.
† Per cent of total phospholipid of this fraction.

TABLE III

Phospholipid composition of fraction with density <1.063

Subject	"Cephalin"		Lecithin		Sphingomyelin		Lysolecithin	
	mM/L.	*% total**	*mM/L.*	*% total**	*mM/L.*	*% total**	*mM/L.*	*% total**
E. R.	0.068	4.6	0.98	66.4	0.37	25.4	0.053	3.6
N. R.	0.047	5.2	0.62	67.9	0.21	22.6	0.040	4.4
H. D.	0.053	5.0	0.70	65.6	0.28	26.4	0.031	2.9
E. Ri.	†	†	1.35	66.6	0.51	25.2	0.089	4.4
A. G.	0.055	4.1	0.89	66.0	0.34	25.1	0.065	4.8
D. G.	0.048	4.4	0.71	64.8	0.29	26.2	0.051	4.7
A. R.	0.051	3.6	0.93	65.3	0.39	27.1	0.058	4.1
R. D.	0.063	3.9	1.10	67.8	0.40	24.6	0.062	3.8
Average	0.056	4.4	0.91	66.3	0.35	25.3	0.056	4.1

* Per cent of total phospholipid of this fraction.
† Sample lost.

tion with density > 1.063. The mean molar lysolecithin-to-lecithin ratio in the fraction with density > 1.063 was almost three times that in the fraction with density < 1.063.

The ratios of free-to-total cholesterol and of cholesterol-to-phospholipid found in the separate fractions were similar to those reported by Bragdon, Havel and Boyle (13), and are not recorded here. Nonphospholipid ester, presumably triglyceride, was present in all of the fractions, but about 90 per cent was present in the fraction with density < 1.063 and 10 per cent in the fraction with density > 1.063.

The following three density fractions of serum from an apparently healthy nonfasting adult male were prepared according to the method of Havel, Eder and Bragdon (5) and chromatographed on silicic acid columns: *1)* < 1.019, *2)* 1.019 to 1.063, and *3)* > 1.063. Column fractions were then analyzed for phospholipid constituents by the use of paper chromatography. Since the acid hydrolysate of the second peak material (lecithin) of whole serum extract showed only one major constituent by paper chromatography (*2*), this peak material was not analyzed in the individual density fractions. Acid hydrolysates of the first peak material ("cephalin") of each density fraction and of the first peak material of whole serum extract (*2*) produced similar chromatograms. By far the predominating ninhydrin-reacting spot had the same Rf as ethanolamine. Fainter ninhydrin-reacting spots were evident, one with the Rf of serine, but no spots staining with the phosphomolybdic acid method were found. Acid hydrolysates of the "cephalin" peak material of each of the fractions also showed a spot with the Rf and staining properties of inositol.

Acid hydrolysates of the third peak material (sphingomyelin) of each of the three fractions likewise produced chromatograms similar to the third peak material acid hydrolysate of whole serum extract (*2*), showing two spots staining

TABLE IV

Phospholipid composition of fraction with density >1.063

Subject	"Cephalin"		Lecithin		Sphingomyelin		Lysolecithin	
	mM/L.	*% total**	*mM/L.*	*% total**	*mM/L.*	*% total**	*mM/L.*	*% total**
E. R.	0.063	4.8	0.86	67.4	0.17	12.9	0.19	14.8
N. R.	0.061	4.5	0.94	69.2	0.16	11.9	0.20	14.5
H. D.	†	†	1.02	71.0	0.21	14.4	0.12	8.3
E. Ri.	0.110	5.9	1.32	71.1	0.22	11.9	0.21	11.1
A. G.	0.080	5.0	1.11	69.6	0.21	13.1	0.19	12.2
D. G.	0.106	6.0	1.19	67.0	0.27	15.1	0.21	11.9
A. R.	0.079	4.2	1.39	73.6	0.28	14.9	0.14	7.2
R. D.	0.117	5.9	1.40	70.4	0.28	14.3	0.19	9.4
Average	0.088	5.2	1.15	69.9	0.23	13.6	0.18	11.2

* Per cent of total phospholipid of this fraction.
† Sample lost.

TABLE V

Molar phospholipid ratios in density fractions

Subject	Sphingomyelin/Lecithin		"Cephalin"/Sphingomyelin	
	<1.063	>1.063	<1.063	>1.063
E. R.	0.38	0.19	0.18	0.37
N. R.	0.33	0.17	0.23	0.38
H. D.	0.40	0.20	0.19	
E. Ri.	0.38	0.17		0.49
A. G.	0.38	0.19	0.16	0.38
D. G.	0.40	0.23	0.17	0.40
A. R.	0.41	0.20	0.13	0.28
R. D.	0.36	0.20	0.16	0.41
Average	0.38	0.19	0.17	0.39

with the phosphomolybdic acid method, one with the mobility of choline and a much smaller one, which is unidentified, and several minor ninhydrin-reacting components.

Paper chromatography of acid hydrolysates of the last peak material (lysolecithin) of each of the three fractions showed one spot with the phosphomolybdic acid stain with the mobility of choline and several lesser ninhydrin-reacting components. These ninhydrin-reacting components were relatively more prominent than those seen with acid hydrolysates of the last peak material from whole serum extract, perhaps due to heavier nitrogenous contamination. To obtain further evidence that the last peak material was composed predominantly of lysolecithin, the unhydrolyzed last peak material of each fraction was also chromatographed on silicic acid-impregnated filter paper and showed a pattern similar to that of the last peak material of whole serum extract (2), *i.e.*, three spots staining with the phosphomolybdic acid method, the major one with the Rf of lysolecithin and two much smaller ones with the Rf's of lecithin and sphingomyelin, respectively.

DISCUSSION

The ultracentrifugation method employing flotation for the separation of serum lipoprotein fractions was used because of its relative simplicity and accuracy. The composition of the lipoprotein fractions of human serum obtained by this method and by other methods has been compared (5, 14). On paper electrophoresis, the fraction with density < 1.063 migrates as beta globulin and the fraction with density > 1.063 as alpha globulin (5). On zone electrophoresis, however, the fraction with density < 1.018 has been found to migrate as alpha$_2$ globulin, although material migrating as alpha$_2$ globulin has also been found in some instances in higher density fractions (15).

Chromatographic analysis of three fractions, with densities of < 1.019, 1.019 to 1.063, and > 1.063, for the individual phospholipids in the present study revealed that all of the phospholipids identified were present in each of the three fractions. For example, acid hydrolysis of the "cephalin" produced ethanolamine, inositol and minor ninhydrin-reacting components in approximately the same relative amounts in each density fraction. Since each density fraction may contain a mixture of lipoproteins, however, the possibility that different lipoproteins contain different mixtures of phospholipids or even single phospholipids cannot be excluded.

When comparing the density fractions with regard to the relative quantities of the individual phospholipids present, however, definite differences were apparent. The values in the fractions with density < 1.019 and < 1.063 were quite similar but differed from those in the fraction with density > 1.063. These differences were noted only for sphingomyelin and lysolecithin, as the relative amounts of "cephalin" and lecithin in the fractions with density < 1.063 and > 1.063 were similar. The molar sphingomyelin-to-lecithin ratio in the fraction with density < 1.063 was two times that in the fraction with density > 1.063, with little variation between subjects (Table V). The molar "cephalin"-to-sphingomyelin ratio in the fraction with density < 1.063, on the other hand, was approximately one-half that found in the fraction with density > 1.063, indicating that the molar "cephalin"-to-lecithin ratio was similar in both fractions. The molar lysolecithin-to-lecithin ratio in the fraction with density > 1.063 was almost three times that in the fraction with density < 1.063, and this heavier fraction contained about three-fourths of the total serum lysolecithin. It has been found, moreover, that most of the lysolecithin in the fraction with density > 1.063 is concentrated in a fraction with density > 1.210 (16). The molar "cephalin"-to-sphingomyelin and lysolecithin-to-lecithin ratios showed greater variation between subjects possibly because of the larger errors involved in the determination of "cephalin" and lysolecithin.

This difference in sphingomyelin-to-lecithin ratio in the two large groups of serum lipoproteins has been noted by Steele and Kayden (17). Using electrophoresis on starch and determining the individual phospholipid contents by differential hydrolysis, these investigators found that the molar sphingomyelin-to-lecithin ratio was three times as great in the β- as in the α-lipoprotein fraction. The markedly greater variability in this ratio between subjects in their study as compared to the present study, however, suggests that their methods were less accurate.

The free-to-total-cholesterol and cholesterol-to-phospholipid ratios found in the three density fractions were similar to those reported by Bragdon, Havel and Boyle (13). About 90 per cent of the total nonphospholipid ester, presumably triglyceride, was found in the fraction with density < 1.063 and 10 per cent in the fraction with density > 1.063.

SUMMARY

Serum lipoprotein fractions of density < 1.019, 1.019 to 1.063, and > 1.063 from an apparently healthy nonfasting adult male were separated by ultracentrifugation and analyzed for the individual phospholipids using chromatography on both silicic acid columns and paper. All of the phospholipids and possible phospholipid constituents identified were found in each of the three fractions.

Serum lipoprotein fractions of density < 1.019, < 1.063 and > 1.063 from eight apparently healthy fasting adults were separated by ultracentrifugation and analyzed for the individual phospholipids, cholesterol, cholesterol ester and triglyceride, using chromatography on silicic acid columns. The relative proportions of the individual phospholipids in the fractions with density < 1.019 and < 1.063 were similar but differed from the values found in the fraction with density > 1.063. In the fraction with density < 1.063, the molar ratio of sphingomyelin-to-lecithin was twice, and of "cephalin"-to-sphingomyelin about one-half, the corresponding value found in the fraction with density > 1.063. Both fractions, therefore, had similar molar "cephalin"-to-lecithin ratios. The molar lysolecithin-to-lecithin ratio of the heavier fraction was almost three times that of the lighter fraction, but there was much variation between subjects.

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THE OXYGEN CONSUMPTION AND EFFICIENCY OF THE RESPIRATORY MUSCLES IN HEALTH AND EMPHYSEMA *

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The work of moving the lung, chest wall, diaphragm and abdominal contents during spontaneous respiration is performed by the muscles of respiration. In order to obtain a better understanding of the disability present in pulmonary disease it would be advantageous to have, in normal and diseased states, a measure of the total work being done, the energy cost of performing this work and the efficiency of the muscles of respiration.

There are few measurements of total mechanical work and efficiency of the respiratory muscles in the literature (1). A simple method for the determination of the oxygen consumption and efficiency of the respiratory muscles, as well as an indirect measurement of the total mechanical work, has recently been described (2). Reproducible results were obtained in both a normal and an emphysematous subject intensively studied.

The purpose of this paper is to report further data on the oxygen cost, efficiency and the total mechanical work of the respiratory process in a series of normal and emphysematous subjects.

METHODS

Subjects. Table 1 lists the physical characteristics of the 16 normal and 22 emphysematous subjects studied. The normal subjects had no clinical or radiological evidence of cardio-respiratory disease. The diagnosis of pulmonary emphysema was based on the clinical history and examination, and on the radiological and spirographic evidence. Vital capacities and maximum breathing capacities were measured on a Collins respirometer with valves and carbon dioxide absorber removed. The maximum of at least four determinations was chosen. All patients with emphysema showed marked obstruction to expiratory outflow and reduction of the maximum breathing capacity.

Oxygen consumption of the respiratory muscles. The oxygen consumption was determined at rest and at several levels of increased ventilation by a modification of a method previously described (2). The apparatus employed is illustrated in Figure 1. The subject breathed into and out of a 9 L. Collins respirometer with carbon dioxide absorber incorporated. A cam on the pulley of the spirometer activated a micro-switch which in turn opened and closed a solenoid valve through which oxygen could be delivered from a second spirometer. The micro-switch was activated towards end-inspiration so that a nonsloping record of tidal volumes and the integrated minute ventilation were obtained on the first spirometer, while the oxygen being consumed was recorded on the second spirometer.

All subjects were studied in the sitting position, having fasted for at least nine hours and having rested in a comfortable chair for one hour prior to the study. All breathed 100 per cent oxygen from a meteorological balloon for 10 minutes and into and out of the spirometer circuit for five to seven minutes before any measurements were taken. This was done because it was found impossible to obtain reproducible measurements within a shorter time. Seven to 10 minute measurements of oxygen consumption and ventilation were made at rest and during increased ventilation. Oxygen consumption was expressed in milliliters per minute S.T.P.D. and ventilation in liters per minute B.T.P.S.

At least two levels of increased ventilation were achieved by the interposition of dead spaces consisting of thick-walled rubber tubing (2.5 cm. internal diameter) between

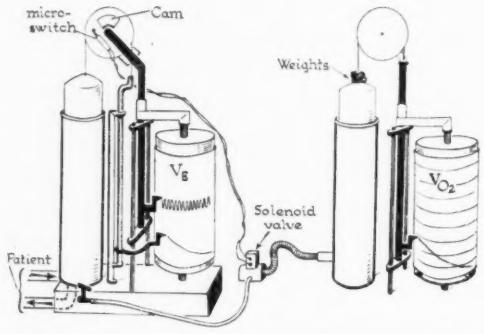

FIG. 1. THE APPARATUS EMPLOYED TO MEASURE MINUTE VENTILATION AND OXYGEN CONSUMPTION IN ORDER TO CALCULATE THE OXYGEN CONSUMPTION AND EFFICIENCY OF THE RESPIRATORY MUSCLES

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† Markle Scholar in Medical Science.

TABLE I

Physical characteristics and oxygen cost of increased ventilation in normal and emphysematous subjects

Cond.	Subj.	Age	Sex	Height	B.S.A.	Vital capacity		Max. breath. cap.		O_2 cost*
						Observed	Predicted	Observed	Predicted	
		yrs.		*cm.*	$M.^2$	*ml.*	*%*	*L./min.*	*%*	*ml./L.*
Normal	1	25	M	185	2.10	5,890	128	210	139	0.76
	2	43	M	171	1.85	4,760	125	140	130	1.10
	3	32	M	180	1.87	6,200	152	182	152	0.78
	4	21	M	168	1.80	4,650	138	159	132	1.62
	5	43	M	170	1.80	4,250	119	104	96	1.12
	6	25	M	190	2.22	6,180	130	174	124	1.21
	7	36	M	203	2.50	6,940	128	202	139	1.34
	8	21	M	197	2.15	6,020	118	208	115	1.38
	9	30	F	159	1.63	3,460	117	102	113	1.78
	10	22	M	182	1.97	4,950	110	159	115	1.87
	11	21	M	186	1.95	6,260	133	224	159	1.49
	12	21	M	177	2.03	5,320	122	159	119	1.23
	13	24	F	159	1.55	3,450	117	139	153	1.15
	14	21	F	160	1.58	3,363	109	119	117	0.45
	15	36	F	169	1.78	4,529	136	101	107	0.77
	16	27	F	164	1.70	3,470	111	126	133	0.58
Emphysema	17	71	M	168	1.59	2,910	90	26	33	2.50
	18	56	M	170	1.65	3,870	108	37	35	7.27
	19	68	M	173	1.67	2,740	80	44	52	9.20
	20	61	M	168	1.62	1,710	50	28	32	3.70
	21	38	M	168	1.62	2,010	53	25	22	3.33
	22	71	M	150	1.36	1,780	68	22	34	6.70
	23	50	M	154	1.94	2,465	82	39	45	2.80
	24	59	M	174	1.72	1,090	30	14	15	8.12
	25	51	M	173	1.74	2,840	77	48	48	3.50
	26	56	M	156	1.69	3,035	100	64	78	1.68
	27	59	M	170	1.49	2,310	66	17	19	8.00
	28	72	M	162	1.64	2,120	71	28	38	3.68
	29	66	M	167	1.65	2,650	81	30	37	9.51
	30	60	M	168	1.85	2,450	72	35	39	4.43
	31	59	M	157	1.68	2,445	81	31	39	4.03
	32	73	M	175	1.87	2,820	81	46	55	5.17
	33	69	M	179	2.00	1,875	51	20	22	7.84
	34	61	M	165	1.62	3,250	99	30	35	18.50
	35	55	M	169	1.98	2,521	71	56	59	3.86
	36	64	M	173	1.86	2,220	62	36	40	8.02
	37	54	M	168	1.78	2,350	67	23	24	7.90
	38	47	M	167	1.78	2,570	71	28	28	7.45

* Oxygen cost of an increase in ventilation of 6 to 10 L. per minute from resting.

the subject and the spirometer, the smaller of the dead spaces producing an increase in ventilation of 6 to 10 L. per minute, and the larger from 12 to 25 L. per minute. The resistance of the whole dead space-spirometer system was less than 1 cm. H_2O per L. per second at a flow rate of 1.5 L. per second.

Subjects were allowed to increase their ventilation spontaneously with no control of rate or depth while breathing through a dead space. The sequence in which the various increased levels of ventilation were studied was altered at random. Observations at rest with no added dead space were made at the beginning and end of each series of measurements. Fifteen minutes of rest was allowed between observations, during which time room air was breathed.

For each subject the difference in oxygen consumption at the resting ventilation and at a ventilation 6 to 10 L. above the resting ventilation was divided by the difference in ventilations and expressed as the oxygen cost of increased ventilation in milliliters per liter of ventilation.

Efficiency of the respiratory muscles. The efficiency of the respiratory muscles was determined in nine normal and seven emphysematous subjects by measuring the extra oxygen consumption associated with the performance of a known added respiratory work load, using the respirometer as described above. Work was added by having the subject inspire through metal tubing (2.5 cm. internal diameter) projecting down under a water seal (2). This produced a constant additional alveolar spirometer pressure difference throughout inspiration, relatively independent of the rate of air flow, and left expiration unimpeded. The time taken for this procedure was identical to that for measuring the oxygen consumption of the respiratory muscles. The added work was calculated by multiplying the minute ventilation

TABLE II

Oxygen cost and efficiency of the respiratory muscles, determined by the open-circuit and closed-circuit techniques

Test condition	Subject No. 2				Subject No. 3			
	Respiratory quotient	Added work	O_2 cost	Effic.	Respiratory quotient	Added work	O_2 cost	Effic.
		Kg. M.	*ml./L.*	%		*Kg. M.*	*ml./L.*	%
Open-circuit	0.87		1.35		0.80		1.12	
	0.85		0.76		0.76	4.40		7.00
	0.76	5.48		8.50	0.95		0.96	
	0.82		0.84		1.01	4.27		7.56
	0.71	5.51		7.72				
Closed-circuit			1.20				0.75	
		4.78		7.65		5.98		7.12
			1.01				0.86	
		5.36		7.25		4.08		8.08
			1.12				0.92	

in liters by the added pressure in centimeters H_2O by 10^{-4} and was expressed in kilogram-meters per minute. The extra oxygen consumption associated with the added work load was converted to its energy equivalent assuming a respiratory quotient of 0.82 and expressed in kilogram-meters per minute. The added mechanical work, divided by the energy equivalent of the extra oxygen consumption, yielded the efficiency of the respiratory muscles for handling work loads.

In four normal subjects and three patients with emphysema the efficiency was determined while breathing with three to four levels of added work. The normals were studied with added work loads of from 2.42 to 6.56 Kg. M. per minute, while the patients were studied with added work loads of from 0.54 to 2.34 Kg. M. per minute. The range of values obtained for per cent efficiency in any one subject was no greater than one. The mean of the determinations in each subject was used in this report.

In order to test the validity of the "closed-circuit" method, the oxygen consumption and efficiency of the respiratory muscles were determined with an "open-circuit" technique in two subjects (Nos. 2 and 3). Oxygen consumption was measured at rest and while breathing 5.73 per cent CO_2 in air with and without an added inspiratory resistance as outlined above. Expired air was collected in a 300 L. Tissot spirometer and analyzed with a Scholander micro-gas analyzer. Table II compares the oxygen cost and efficiency of the respiratory muscles determined by the "open-circuit" and "closed-circuit" techniques in the two subjects. It can be seen that the values obtained by the two techniques compared favorably.

Total mechanical work of breathing. Knowledge of the energy equivalent of the oxygen cost of breathing and of the efficiency allows calculation of the total mechanical work of breathing at any level of ventilation. The oxygen cost of breathing and efficiency for handling the added work loads can be determined as described above. Our data and that reported previously (2) indicate that the efficiency of the respiratory muscles for handling added work remains essentially unchanged over a large range of added work loads, suggesting that the value for efficiency measured under such conditions may be applied to situations where there is no added work load. Therefore, knowledge of the efficiency and the oxygen cost of breathing at the resting ventilation allows calculation of the total mechanical work of breathing at rest.

In this report the total mechanical work of breathing per liter of ventilation at rest was calculated from the product of the efficiency and the energy equivalent of the oxygen cost of breathing per liter of ventilation at rest and expressed in kilogram-meters per liter of ventilation. This value, multiplied by the resting minute ventilation in liters, yielded the total mechanical work of breathing per minute at rest.

RESULTS

The oxygen cost of increased ventilation in the normal and emphysematous subjects is shown in Table I. It can be seen that the oxygen consump-

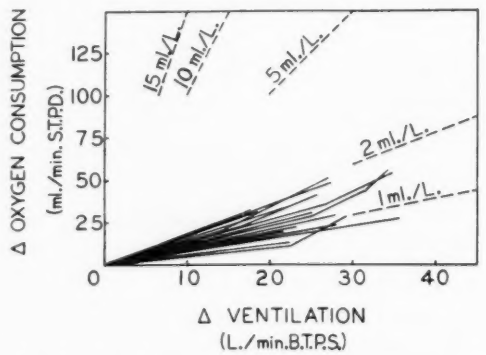

FIG. 2. THE CHANGE IN OXYGEN CONSUMPTION ASSOCIATED WITH CHANGE IN MINUTE VENTILATION IN 16 NORMAL SUBJECTS

Also shown are isopleths representing the oxygen cost of breathing per liter of ventilation.

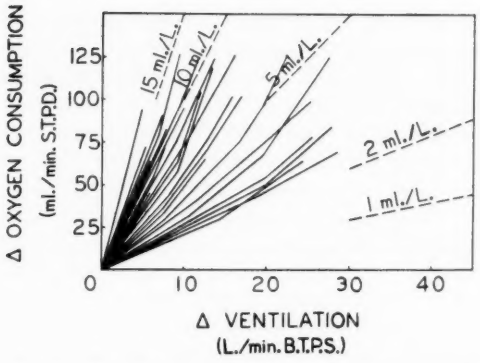

FIG. 3. THE CHANGE IN OXYGEN CONSUMPTION ASSOCIATED WITH CHANGE IN MINUTE VENTILATION IN 22 PATIENTS WITH EMPHYSEMA

Also shown are isopleths representing the oxygen cost of breathing per liter of ventilation.

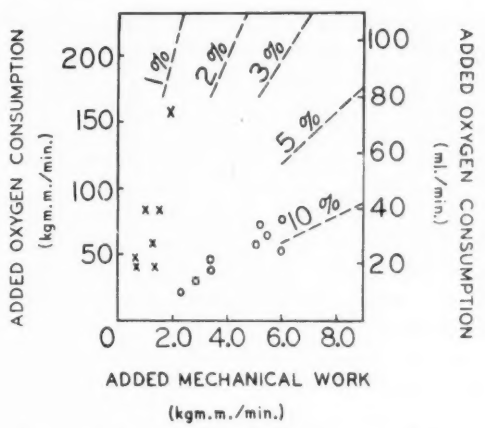

FIG. 4. THE EXTRA OXYGEN CONSUMPTION REQUIRED TO HANDLE ADDED RESPIRATORY WORK LOADS IN NINE NORMAL AND SEVEN EMPHYSEMATOUS SUBJECTS

Also shown are isopleths representing the efficiency of the respiratory muscles for handling added work loads. ○ represents normal subjects; × represents emphysematous subjects.

tion of the respiratory muscles per liter of ventilation was considerably higher in the patients with emphysema than in the normal subjects. The cost ranged between 0.45 to 1.87 ml. per L. of ventilation (mean 1.16) in the normal subjects, while it ranged between 1.68 and 18.50 ml. per L. of ventilation (mean 5.96) in the emphysematous subjects.

In Figures 2 and 3 the increments in oxygen consumption associated with changes in ventilation in the normal subjects and patients with emphysema are shown. It can be seen that in the normal subjects the oxygen cost of an increase in ventilation of 30 L. per minute above the resting ventilation was less than 2 ml. per L. On the other hand, in the subjects with emphysema the oxygen cost was high for even slight increases in ventilation and rose still further when ventilation was increased.

The extra oxygen consumption associated with added work loads in nine normal subjects and seven patients with emphysema is shown in Figure 4. It can be seen that the extra oxygen consumption per unit added work load was greater in the patients with emphysema than in the normals.

In Table III the calculated efficiencies of the respiratory muscles of the nine normal and seven

TABLE III

Total mechanical work and efficiency of the respiratory muscles in normal and emphysematous subjects

Normal				Emphysematous			
Subj.	Efficiency	Total mechanical work at rest		Subj.	Efficiency	Total mechanical work at rest	
	%	*Kg. M./L.*	*Kg. M./min.*		%	*Kg. M./L.*	*Kg. M./min.*
1	10.80	0.173	1.52	17	2.74	0.141	1.39
2	7.45	0.172	1.38	18	1.45	0.203	1.77
3	7.60	0.123	0.82	19	2.30	0.159	1.39
4	8.20	0.280	2.69	20	2.04	0.120	0.83
5	11.10	0.326	3.00	21	1.24	0.188	1.20
6	8.04	0.204	2.74	22	1.47	0.284	2.68
7	7.20	0.203	1.83	23	1.60	0.123	1.22
8	7.43	0.215	1.89				
9	9.35	0.349	2.27				
Mean	8.58	0.227	2.02		1.83	0.174	1.49

emphysematous subjects are presented. The efficiency of the respiratory muscles of the normal subjects ranged between 7.20 and 11.10 per cent (mean, 8.58 per cent) while the efficiency of the emphysematous subjects was considerably less, ranging between 1.24 and 2.74 per cent (mean, 1.83 per cent).

Also shown in Table III are calculations of total mechanical work of breathing at rest. It can be seen that the total mechanical work of breathing tended to be less in patients with pulmonary emphysema than in the normal subjects, whether expressed as work per liter of ventilation or work per minute.

DISCUSSION

The data presented indicate that the use of a "closed-circuit" technique yields values for oxygen cost of breathing and efficiency of the respiratory muscles which are comparable to those obtained by the "open-circuit" technique.

It is recognized that values obtained for oxygen consumption or efficiency of the respiratory muscles for handling added work loads will depend upon the resistance offered by the apparatus used. The values reported in this paper, therefore, are not absolute but do serve to distinguish distinctly between normal and emphysematous subjects.

The values for the oxygen consumption of the respiratory muscles in the normal individuals studied are similar to those reported by previous investigators (1–4) but those for efficiency are slightly higher than previously supposed (1).

The results reported in this study indicate that in the emphysematous subjects the oxygen consumption of the respiratory muscles is considerably higher than in the normals. In addition, small increases in ventilation are associated with marked increases in oxygen consumption in the emphysematous patient. These findings are comparable to those previously reported (2, 4). The efficiency of the respiratory muscles in the emphysematous subjects is considerably lower than in the normals. This is in disagreement with the findings of Fritts and Cournand (5) but it must be pointed out that they measured work done on the lungs only.

Although total mechanical work has been measured while the subject was ventilated in a respirator (6, 7), there is at present no method available for measuring the total mechanical work of breathing during spontaneous respiration. The method described in this paper may yield an indirect measurement. The values for total work found in normal individuals are considerably higher than has previously been supposed (6). However, previous measurements of total work were no greater than that of work done on the lungs alone (8, 9) and were probably, therefore, an underestimation.

The data obtained suggest that the total mechanical work performed on lung and thorax tends to be less in the emphysematous than in normal individuals at low ventilations. This might be expected since about 63 per cent of the work of breathing is performed in overcoming elastic resistances (6) and a substantial loss of lung elasticity occurs in emphysema (8).

Though the total mechanical work done on lungs and thorax tends to be less at low ventilations in patients with emphysema than in normals, the oxygen cost of breathing is four to five times greater because of the markedly reduced efficiency of the respiratory muscles. With increases in ventilation there is a disproportionate increase in oxygen consumption of the respiratory muscles in emphysema. This may help to explain the disability present in pulmonary emphysema and the inability of the severely emphysematous patient to meet the increased energy demands of exercise and infection.

SUMMARY AND CONCLUSIONS

1. The oxygen consumption of the respiratory muscles was measured in 16 normal and 22 emphysematous subjects. The oxygen cost of increased ventilation was considerably higher in the emphysematous subjects and rose even further with slight increases in ventilation.

2. The efficiency of the respiratory muscles was determined in nine normal and seven emphysematous subjects. The efficiency was considerably lower than the normal in the patients with emphysema.

3. The total mechanical work at rest, measured indirectly, tended to be less than the normal in the patients with emphysema, whether expressed as work per minute or work per liter of ventilation.

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HYPOVENTILATION IN OBESITY*

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Recently, numerous reports have noted that marked obesity may be associated with hypoxia, hypercapnia, polycythemia and right heart failure in the absence of apparent intrinsic pulmonary disease. Weight loss has been followed by alleviation of the derangements in some cases (1–4) and this syndrome has been called the "Pickwickian Syndrome" by Burwell, Robin, Whaley and Bickelmann (5).

Fairly general agreement exists that the basic physiological defect in this syndrome is alveolar hypoventilation which leads to hypoxia and hypercapnia followed by polycythemia and eventual cor pulmonale. It has been suggested that the alveolar hypoventilation in obesity is due to an increased resistance to respiratory movements, thus necessitating an increase in work per unit ventilation (*2*, 6–8), although little evidence to support this has been advanced. In addition, there is little information in the literature regarding pulmonary function and blood volume in obese individuals.

The purposes of this paper are *1*) to report studies of pulmonary function and blood volume in a group of obese subjects, *2*) to attempt to correlate arterial blood gases with the blood volume measurements, *3*) to report measurements of the oxygen cost of breathing in these individuals, and *4*) to attempt to correlate the oxygen cost of breathing with the occurrence of alveolar hypoventilation.

METHODS

Twenty-six obese individuals, whose physical characteristics are shown in Table I, were studied. The mean age was 46 years; the mean weight, 113 Kg.; and mean heights and body surface areas, 168 cm. and 2.18 M.^{2}, respectively.

Any patient giving a history of lung disease such as chronic cough and sputum, cardiac disease or neuromuscular disease was excluded from the series. Some patients had complaints of somnolence and exertional dyspnea but all were primarily referred because of their obesity.

Ventilatory function was assessed in 21 subjects. Vital capacities, timed vital capacities and maximum breathing capacities were measured using a Collins respirometer with the valves and CO_2 absorber removed and a high speed rotating drum incorporated. The maximum of at least three trials was recorded. Predicted values were determined from the data of Baldwin, Cournand and Richards (9).

The intrapulmonary distribution of inspired gas was assessed by determining the breath-to-breath washout of nitrogen from the lungs while breathing oxygen and by measurement of the concentration of nitrogen in alveolar gas after seven minutes of the oxygen breathing, using an instantaneously-recording nitrogen meter (10).

Simultaneous samples of arterial blood and expired air were obtained in 15 subjects and arterial blood alone in another three. Collections were made after the individuals had been in the semirecumbent position for 20 to 30 minutes. Arterial carbon dioxide and oxygen tensions were determined by a modification (11) of the technique of Riley, Proemmel and Franke (12). Expired air was collected over a three minute period in a Tissot spirometer and analyzed with the micro-Scholander apparatus (13). From these data tidal volume and physiological dead space, calculated by the Bohr method (14), were obtained.

Blood volumes were determined in 16 subjects using the slightly modified (15) T-1824 dye dilution technique of Gibson and Evans (16). These studies were done after the subjects had fasted for 12 hours and just before the collection of arterial bloods and expired gas. All samples of blood were drawn from an indwelling arterial needle.

The oxygen cost of the respiratory mechanism was determined in 25 subjects using the method of Campbell, Westlake and Cherniack (17) as modified by Cherniack (18). The oxygen consumption at rest and at increased ventilation was measured, using a closed-circuit spirometer in which a carbon dioxide absorber was incorporated. A cam on the pulley of the spirometer activated a micro-switch, which in turn controlled a solenoid valve, through which oxygen was delivered from a second spirometer. It was thus possible to keep a level, non-sloping baseline on the records of ventilation while the second spirometer recorded the oxygen consumed.

In order to obtain oxygen consumption at different levels of ventilation, the ventilation was altered by the

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interposition of thick-walled rubber tubing, 2.5 cm. internal diameter, between the subject and the spirometer circuit. Tubes of different lengths were chosen in random order and the individuals were allowed to increase their ventilation spontaneously with no external control of rate or depth of breathing.

Before studies were begun, the subjects, who had fasted for at least nine hours, rested in a comfortable chair for one hour in the case of out-patients and for 20 minutes in the case of hospitalized patients.

Resting oxygen consumption and ventilations were recorded at the beginning and at the end of each study and the mean was used for calculation. Before each measurement, whether resting or at increased ventilation, the subjects breathed oxygen from a meteorological balloon for 10 minutes and then into and out of the oxygen-filled spirometer circuit for five minutes in order to allow the attainment of a steady state. Recordings were then taken over a 5 to 10 minute period. Oxygen consumption was corrected to S.T.P.D. and ventilation to B.T.P.S. Ten to 20 minutes rest was allowed between each measurement.

In each subject, oxygen consumptions at the resting ventilation and at at least two increased ventilations were obtained, one of the increased ventilations being 7 to 13 L. per minute above the resting. The difference between the oxygen consumption at the increased ventilation and at rest, divided by the difference in ventilation, has been used as the oxygen cost of increased ventilation and expressed in milliliters of oxygen per liter of ventilation.

In three subjects the oxygen cost of increased ventilation, as calculated above, was determined on several occasions and in each did not vary by more than 0.3 ml. O_2 per L.

RESULTS

1. Ventilatory function

The results of the ventilatory function studies are listed in Table I. It will be seen that some subjects had fairly marked reduction in vital capacity and maximum breathing capacity. However, timed vital capacities and maximum mid-expiratory flow rates performed before and after nebulized bronchodilator revealed no evidence of bronchiolar obstruction.

The index of intrapulmonary mixing was normal in all subjects but the washout of N_2 from the lungs was somewhat slower than normal in Subjects 18, 20, 23 and 25.

TABLE I

Physical data and pulmonary function in obese subjects

Subject no., sex	Age	Height	Weight	Body surface area	Vital capacity		Maximum breathing capacity		I.I.P.M.*	D.S./T.A.†	O.C.I.V.‡
	yrs.	*cm.*	*Kg.*	*M.²*	*ml.*	*% of pred.*	*L./min.*	*% of pred.*	*% N_2*	*%*	*ml./L.*
1, F	46	163	100	2.04							5.76
2, F	46	162	111	2.13							4.45
3, F	53	165	90	1.97							2.5
4, F	31	174	114	2.26							2.57
5, F	47	160	97	1.98	3,420	129	93	113	0.4		9.73
6, F	53	163	99	2.03	2,355	86	74	75	0.4		1.75
7, F	35	162	101	2.02	3,810	131	154	174	0.4		0.82
8, M	37	180	131	2.47	4,500	107	124	103	0.4		5.24
9, F	38	151	115	2.07	2,095	82	69	88	0.4		1.75
10, F	69	155	101	1.94	1,875	88	56	84	0.4		1.75
11, M	56	166	86	1.95	3,920	114	127	140	0.4	23	1.86
12, F	51	170	127	2.33	3,260	111	79	89	0.4	22	3.47
13, F	47	156	103	2.00	2,650	104	71	92	0.4	32	2.57
14, M	43	175	123	2.44	4,650	119	166	151		32	1.80
15, F	28	178	124	2.40	3,670	109	110	108	0.4	26	2.77
16, M	36	185	135	2.55	6,030	136	129	98	0.4	37	2.70
17, M	64	174	114	2.26	3,380	98	82	90	0.4	42	2.01
18, M	61	182	111	2.36	4,120	112	122	123	2.0	61	3.93
19, F	52	155	122	2.14	2,930	122	101	120	0.4	43	2.3
20, F	44	163	98	2.02	2,900	104	72	88	0.4	33	1.6
21, M	41	175	120	2.31	3,820	93	104	90		44	2.76
22, M	45	173	121	2.31						43	3.85
23, F	49	155	88	1.87	2,260	70	73	83	0.4	52	4.77
24, M	33	173	167	2.65	2,970	75	65	55	0.7	53	6.46
25, F	57	153	125	2.12	1,277	56	52	59	0.4	35	7.04
26, M	34	180	99	2.19	5,260	122	185	145	1.0		

* Index of intra-pulmonary mixing.
† Physiological dead space-tidal air ratio.
‡ Oxygen cost of increased ventilation.

TABLE II

Blood studies in obese subjects

Subject	pO_2	pCO_2	Ht.*	R.C.M./M.²†	P.V./M.²‡	T.B.V./M.²§
	mm. Hg	*mm. Hg*	%	*ml.*	*ml.*	*ml.*
9	94	39	43	1,053	1,378	2,431
10	74	40	44	1,177	1,544	2,721
11	90	31	46			
12	93	40	42	1,105	1,550	2,655
13	116	41	45	1,202	1,463	2,665
14	99	38	51			
15	102	36	38	1,271	1,940	3,210
16	79	39	47	1,269	1,460	2,729
17	64	39	48	1,612	1,780	3,392
18	65	45	45	1,529	1,530	3,059
19	76	44	43	1,178	1,586	2,764
20	83	40	37	926	1,555	2,481
21	67	44	49	1,330	1,385	2,715
22	65	47	44	1,595	1,935	3,530
23	75	53	42	1,110	1,616	2,726
24	51	77	53	1,966	1,715	3,681
25	44	57	44	1,588	2,038	3,626
26	71	40	60	1,545	1,195	2,740

* Hematocrit.
† Red cell mass per square meter body surface area.
‡ Plasma volume per square meter body surface area.
§ Total blood volume per square meter body surface area.

2. Physiological dead space-tidal air ratios

Dead space-tidal air ratios are also listed in Table I. This ratio was greater than the normal upper limit of 30 per cent (19) in 12 of the 15 obese subjects in whom it was measured.

3. Arterial blood studies

Table II lists the arterial blood gas tensions in 18 obese subjects. The subjects can be divided into three groups. In six subjects pO_2 was greater than 90 mm. Hg and pCO_2 was less than 45 mm. Hg; in eight subjects pO_2 was less than 90 mm. Hg and pCO_2 less than 45 mm. Hg; while four subjects had pO_2 less than 90 mm. Hg and pCO_2 greater than 45 mm. Hg.

The tidal volumes, respiratory rates and physiological dead spaces of the 15 subjects on whom these measurements were obtained are shown in Figure 1. Except for one patient with hypoxia, all physiological dead spaces are within normal limits as defined by Comroe and co-workers (19). It will be noted that there was a tendency for the tidal volume to be less in those patients with hypoxia than in those with normal gas tensions and this is more pronounced in those subjects with both hypoxia and hypercapnia. In addition, it will be seen that there was a tendency for the respiratory rate to increase as the tidal volume decreased.

4. Blood volume studies

Table II also lists the partitions of blood volume in 16 obese subjects. The mean blood volume in the male subjects was 3,122 ml. per M.² (S.D., ±412), the mean plasma volume was 1,571 ml.

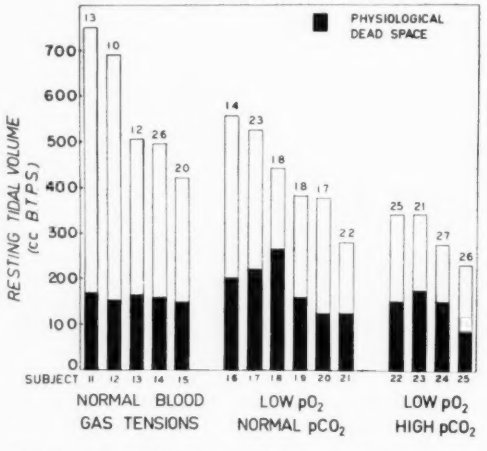

FIG. 1. TIDAL VOLUMES AND PHYSIOLOGICAL DEAD SPACES IN THE THREE GROUPS OF OBESE SUBJECTS
Respiratory rates are also shown for each subject.

TABLE III

The partitions of blood volume in normal and obese individuals

Investigator	Method	Males			Females		
		No. of subjects	P.V.*	R.C.M.†	No. of subjects	P.V.*	R.C.M.†
			ml.	*ml.*		*ml.*	*ml.*
Present study	T-1824	7	1,571 ± 253	1,549 ± 225	9	1,630 ± 217	1,179 ± 182
Doupe, Ferguson and Hildes (15)	T-1824	72	1,645 ± 130				
Gibson and Evans (16)	T-1824	49	1,619 ± 182		39	1,467 ± 160	
Hedlund (20)	P^{32}	35		1,131 ± 131	7		868 ± 115
Samet, Fritts, Fishman and Cournand (21)	T-1824	30	1,527 ± 156		30	1,463 ± 162	
	P^{32}	30		1,039 ± 123	30		782 ± 80

* Mean plasma volume per square meter body surface area plus or minus standard deviation.
† Mean red cell mass per square meter body surface area plus or minus standard deviation.

per $M.^2$ (S.D., ± 253) and the mean red cell mass was 1,549 ml. per $M.^2$ (S.D., ± 225). The mean blood volume in the females was 2,810 ml. per $M.^2$ (S.D., ± 375), the mean plasma volume was 1,630 ml. per $M.^2$ (S.D., ± 217) and the mean red cell mass was 1,179 ml. per $M.^2$ (S.D., ± 182).

In Table III these results are compared with other reported measurements on subjects with predominantly normal weights (15, 20, 21). It can be seen that in the male subjects the mean plasma volume per $M.^2$ apparently was not different from the rest, while in the females it was slightly higher. Red cell mass per $M.^2$ was higher than that reported in the literature in both sexes. It should be noted, however, that the red cell masses reported by Hedlund (20) and by Samet, Fritts, Fishman and Cournand (21) were determined using the tagged red cell method, while in this series it was estimated indirectly by measuring the plasma volume and hematocrit.

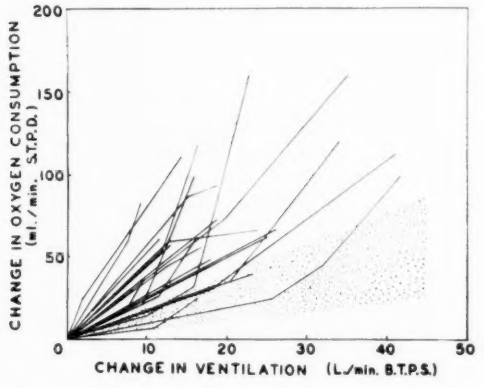

FIG. 2. THE CHANGES IN OXYGEN CONSUMPTION ASSOCIATED WITH CHANGES IN VENTILATION IN 25 OBESE SUBJECTS

The stippled area represents the range found in normal subjects (17).

When an attempt was made to correlate the partitions of blood volume and the resting arterial blood gas tensions, it was found that in the seven male subjects there was a significant correlation between red cell mass and pO_2 ($p < 0.01$) and pCO_2 ($p < 0.05$), and between total blood volume and pO_2 ($p < 0.05$). In the nine female subjects the only significant correlation was between red cell mass and pCO_2 ($p < 0.05$).

5. Oxygen cost of breathing

The oxygen cost of increased ventilation for each subject is given in Table I. These values are to be compared with values ranging from 0.5 to 1.8 ml. O_2 per L. obtained in 16 normal subjects (18). Thus, 18 of the 25 obese subjects had values higher than the upper limit of normal. This can also be seen in Figure 2 which shows the increments in oxygen consumption associated with increases in ventilation. In addition, it can be seen that in most obese subjects there was a disproportionate increase in the oxygen cost of breathing with further increases in ventilation.

The relationship between the oxygen cost of increased ventilation and the resting arterial oxygen and carbon dioxide tensions in the 17 obese subjects who had arterial blood estimations is shown in Figures 3 and 4. It can be seen that there was a tendency for the highest costs of increased ven-

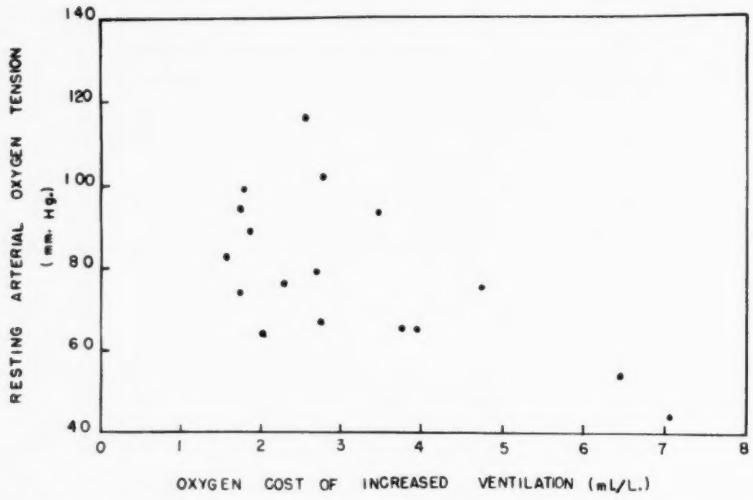

FIG. 3. THE RELATIONSHIP BETWEEN THE RESTING ARTERIAL OXYGEN TENSION AND THE OXYGEN COST OF INCREASED VENTILATION IN 17 OBESE SUBJECTS

tilation to be asociated with the lowest arterial oxygen tensions and the highest arterial carbon dioxide tensions.

In two of the patients with highest carbon dioxide tensions and costs of breathing the physical properties of the lungs were studied. The lung compliance was 0.220 in Subject 24 and 0.300 in Subject 25. The viscous resistance, as expressed by the pressure required to cause a flow of 60 L. per minute, was 1.8 cm. H_2O in Subject 24 and 2.4 cm. H_2O in Subject 25 on expiration, with similar values for inspiration. These measurements are within the normal range (22, 23).

No correlation between the oxygen cost of in-

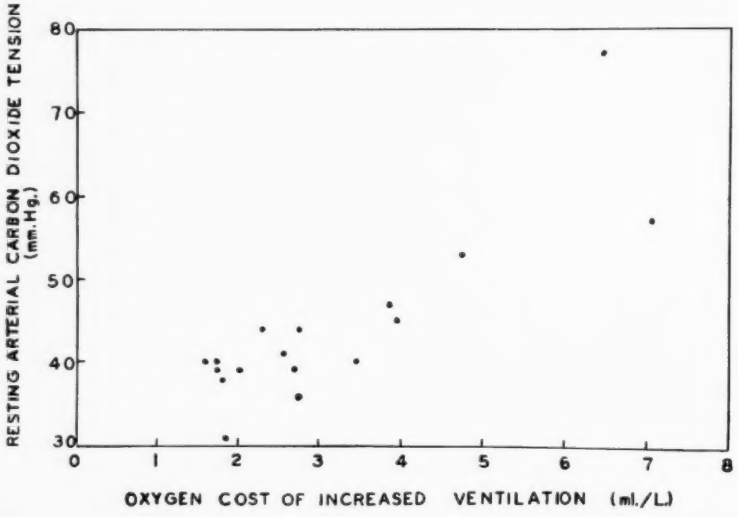

FIG. 4. THE RELATIONSHIP BETWEEN THE RESTING ARTERIAL CARBON DIOXIDE TENSION AND THE OXYGEN COST OF INCREASED VENTILATION IN 17 OBESE SUBJECTS

creased ventilation, measurements of pulmonary function, partitions of blood volume and the degree of obesity could be found in these subjects.

DISCUSSION

It is of some interest to note that, of 18 supposedly normal obese subjects, only 32 per cent had normal resting blood gas tensions. Hypoxia of varying degrees was present in 42 per cent and was associated with hypercapnia in an additional 26 per cent.

Only three of the 12 subjects with hypoxia had hematocrits above 50 per cent but the red cell mass was increased in the majority of the subjects. Although red cell mass was measured indirectly and no correction for body hematocrit was made, it nevertheless appears to be significantly increased. While hypoxia was probably acting as an erythropoietic stimulus in these subjects, its effect is not readily apparent in the hematocrit determination, presumably because of an increase in the plasma volume, particularly in the female subjects.

The correlation between red cell mass and pCO_2 in both sexes may be due to the fact that fairly marked hypoxia always accompanied hypercapnia. No explanation for the increased plasma volume in the obese females studied is readily apparent.

The data presented indicate that the oxygen cost of breathing is increased in obese individuals. If the assumption is made that the oxygen cost of breathing obtained from measurements of small increments in ventilation can be extrapolated back to the resting level, then obese individuals have increased costs of breathing even at rest.

A high oxygen cost of breathing could be due to increased muscular work being done on the lungs or on the thorax or a reduced efficiency of the respiratory apparatus. No measurements of efficiency were made in this study. While slight impairment of distribution of gas may have been present in some of the subjects studied, this could not account for the changes in arterial gases and oxygen cost of breathing. In addition, the absence of bronchiolar obstruction and the lack of clinical history of lung disease suggest that no intrinsic lung pathology was responsible for an increased work of breathing. Also, measurement of the physical properties of the lungs in two of the subjects with high costs of breathing and hypercapnia revealed normal lung compliance and nonelastic resistance. Hence it is postulated that the high cost of breathing is due to increased mechanical work necessary to move thoracic cage, diaphragm and abdominal wall and contents—presumably due to obesity.

The individuals who had the highest oxygen costs of breathing tended to breathe with the lowest tidal volumes and highest respiratory rates at rest. Since Liljestrand (*24*) and Cournand and associates (*25*) have demonstrated that the oxygen cost of breathing is less for a given ventilation when the tidal volume is small, the findings in the obese subjects may be an example of the selection of an optimum rate and depth of breathing where the least work is done.

The rapid, shallow breathing observed in the obese subjects is the type of breathing pattern found when the elastic resistance is increased (*26*), in contra-distinction to the slow, deep breathing encountered when air flow resistance is high (*27*). This suggests that the increased mechanical work necessary to move "the thorax" in these obese subjects was elastic in nature.

In addition, there appears to be a relationship between the oxygen cost of breathing and the arterial carbon dioxide tension in the obese subjects. This is in accordance with the hypothesis advanced by Cain and Otis (*27*) and by Riley (*28*) who viewed respiratory acidosis as an adaptive mechanism sparing oxygen for nonventilatory work, a rise in CO_2 tension being tolerated when the work of breathing is increased "in preference to expending the effort that would be required to keep it at the original level."

The data also indicate that in the obese individual, who may already have an increased cost of breathing at rest, further small increments in ventilation could result in a disproportionate increase in metabolic work of breathing. This would probably be even more exaggerated if an obese person developed bronchitis or other lung disease.

Conversely, in individuals with chronic lung disease and increased work of breathing, the development of obesity would lead to a further rise in the oxygen cost of breathing and possible alveolar hypoventilation. The therapeutic implications of obesity in chronic lung disease thus become apparent.

SUMMARY AND CONCLUSIONS

1. Twenty-six obese subjects were studied. Ventilatory function was assessed in 21, arterial blood gas tensions in 18, blood volume estimations in 16 and the oxygen cost of breathing in 25.

2. Clinical history and ventilatory function revealed no evidence of gross lung disease aside from a slight impairment in distribution in four patients.

3. Twelve of the subjects who had measurements of arterial blood gas tensions had hypoxia. Four had associated hypercapnia which apparently was due to reduced tidal volumes.

4. Red cell masses per $M.^2$ body surface area were increased in both male and female subjects. Plasma volume was increased in the female subjects.

5. The oxygen cost of breathing was increased in the obese subjects.

6. A suggestion has been made that the increased oxygen cost of breathing in the obese subjects was due to an increase in elastic resistance of the thorax.

7. A relationship between the oxygen cost of breathing and the arterial blood tensions of carbon dioxide and oxygen has been indicated.

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STUDIES OF THE INCORPORATION OF Fe^{59} INTO NORMAL AND ABNORMAL HEMOGLOBINS *

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This study was undertaken to ascertain whether different normal and abnormal hemoglobin components exhibit different rates of Fe^{59} incorporation in human subjects and in experimental animals. The hemoglobin of normal man contains minor components, hemoglobins A_2 and A_3, in addition to the principal component, hemoglobin A_1. Patients with hereditary hemoglobin abnormalities have relatively large amounts of abnormal hemoglobins; electrophoretic analysis of the hemoglobin of many experimental animals reveals a small amount of an electrophoretically rapid component, comparable to hemoglobin A_3 in human subjects. The present study was designed to observe the rate of Fe^{59} appearance in at least two hemoglobin components of a subject following the intravenous administration of Fe^{59}.

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MATERIALS AND METHODS

Except for L.B., who had thalassemia major, all the patients in this study had disseminated neoplastic disease in addition to their hemoglobin abnormalities. These patients were selected for study because 40 to 100 μc. of Fe^{59} could be given, and accurate measurement of Fe^{59} activity in small amounts of hemoglobin components was thereby facilitated. Most of the patients received radiotherapy before or during the study period. Two patients (J. B. and L. B.) had received blood transfusions within

TABLE I

Experimental subjects

Pt.	Sex	Age	Diagnosis	Hemoglobin abnormality	Fe^{59} given	Transfusion within 4 mos. of study period	Hematocrit (before injection of Fe^{59})
		yrs.			μc.		%
D. S.	F	80	Chronic-lymphatic leukemia	Sickle cell trait	80	No	30
R. P.	M	21	Osteogenic sarcoma with pulmonary metastases	Sickle cell trait	40	No	45
J. B.	M	49	Carcinoma of the lung	Hemoglobin C trait	100	Yes, 1,500 ml. one month prior to study	34
J. R.	M	65	Multiple myeloma	Alkali resistant hemoglobin (40%)	90	No	26
L. B.	M	30	Cooley's anemia	Alkali resistant hemoglobin	100	Yes	25
S. P.	M	56	Carcinoma of the lung	Rapid abnormal hemoglobin component	100	No	34
O. G.	F	43	Disseminated carcinoma	Sickle cell trait	80	No	32

six weeks before the administration of Fe^{59}. Relevant data concerning these patients are recorded in Table I.

The isotopic iron was administered intravenously as either Fe^{59} citrate or Fe^{59} bound to human β_1 globulin. Blood samples were obtained at intervals after the administration of the isotope and hemoglobin was prepared by the method of Drabkin except that $AlCl_3$ was omitted from the saline washings of the erythrocytes. The final hemoglobin solutions were centrifuged at 15,000 $\times$ G for 30 minutes and converted to carbonmonoxyhemoglobin before electrophoretic or chromatographic separation of components. The hemoglobin of L.B. was separated by alkali denaturation of oxyhemoglobin.

Electrophoretic separation of hemoglobin components was carried out in starch blocks by the method of Kunkel, Ceppellini, Müller-Eberhard and Wolf (1). After a 16 hour run the dense mid-portion of each component was cut from the block, and the carbonmonoxyhemoglobin was eluted from the starch with water. The hemoglobin concentration and isotopic activity were determined on the eluates. The hemoglobin concentration was determined in a Coleman Universal Spectrophotometer and the radioactivity of a 2 ml. aliquot was measured in a well-type scintillation counter. Sufficient counts were recorded to secure an accuracy of at least 3 per cent.

For the separation of fetal hemoglobin, the alkali denaturation technique of Singer, Chernoff and Singer (2) was used in the case of L.B.; the only modification was the use of 0.5 ml. hemoglobin specimens. Alkali denaturation was not entirely satisfactory for the purpose of this study because of the dilution of hemoglobin F in the alkali resistant fraction. Consequently for the second patient with alkali resistant hemoglobin, a modification of the chromatographic technique of Morrison and Cook (3) was utilized for separating fetal hemoglobin. The fetal hemoglobin sample selected for assay was the most

TABLE II

Studies of Fe^{59} incorporation in hemoglobin components of patients with sickle cell trait

Patient	Days after Fe^{59}	Hemoglobin fraction	Radioactivity	Ratio of specific activities of components where total = 100
			cpm/mg. Hgb.	
D. S.	2	Total hemolysate	28	100
		A	27	96
		S	28	100
D. S.	6	Total hemolysate	152	100
		A	159	105
		S	154	101
D. S.	11	Total hemolysate	174	100
		A	176	101
		S	179	103
D. S.	18	Total hemolysate	182	100
		A	182	100
		S	170	93
D. S.	34	Total hemolysate	194	100
		A	196	101
		S	194	100
D. S.	62	Total hemolysate	172	100
		A	169	98
		S	182	106
D. S.	76	Total hemolysate	147	100
		A	170	116
		S	156	106
D. S.	101	Total hemolysate	162	100
		A	161	99
		S	151	93
R. P.	6	Total hemolysate	11.7	100
		A	11.9	102
		S	13.4	115
O. G.	2.5	Total hemolysate	91	100
		A_1	89	98
		S	102	112
		A_2	80	88
		A_3	50	55
O. G.	5	Total hemolysate	129	100
		A_1	127	98
		S	125	97
		A_2	114	88
		A_3	82	64
O. G.	9	Total hemolysate	144	100
		A_1	143	99
		S	146	101
		A_2	122	85
		A_3	85	59
O. G.	10	Total hemolysate	143	100
		A_1	142	99
		S	146	102
		A_2	128	90
		A_3	92	64
O. G.	12	Total hemolysate	142	100
		A_1	148	104
		S	139	98
		A_2	138	97
		A_3	107	75
O. G.	24	Total hemolysate	153	100
		A_1	145	95
		S	167	109
		A_2	114	75
		A_3	111	73
O. G.	39	Total hemolysate	150	100
		A_1	159	106
		S	158	105
		A_2	148	99
		A_3	116	77
O. G.	49	Total hemolysate	150	100
		A_1	145	97
		S	145	97
		A_2	160	107
		A_3	129	86

concentrated 5 ml. fraction eluted with phosphate buffer, pH 6.3, and sodium concentration, 65 mEq. per L. The remainder of the hemoglobin was represented by the most concentrated fraction eluted with a buffer of sodium concentration, 425 mEq. per L. The hemoglobin concentration and radioactivity were determined in the same manner as for components separated by starch electrophoresis.

RESULTS

1. *Major fractions of hemoglobin*

a). *Sickle cell trait.* Three patients with sickle cell trait were studied over periods ranging from 10 to 101 days. No significant difference in Fe^{59} incorporation between sickle cell hemoglobin and hemoglobin A_1 was observed in any of the three patients who were studied. Results in these patients are recorded in Table II.

b). *Hemoglobin C trait.* The patient with hemoglobin C trait who was studied had been transfused six weeks prior to the administration of Fe^{59}. The results of the study are recorded in Table III. Since the isotopic activity of hemoglobin A and C differed in the initial specimens obtained from this patient, the reliability of the observation was checked by separating larger amounts of hemoglobin A and C on successive electrophoretic analyses and subsequently preparing recrystallized hemin from each component. The radioactivity of the preparations of hemin recrystallized from the A and C hemoglobin components showed a C:A ratio of 1.25; the pooled hemoglobin components used for the crystallization had a radioactivity ratio for C and A hemoglobins of 1.4.[1] In this patient the relatively higher radioactivity of the hemoglobin C fraction was attributed to dilution of the hemoglobin A fraction by the hemoglobin of previous transfusions. This hypothesis was sup-

TABLE III

Studies of Fe^{59} incorporation in hemoglobin components of a previously transfused patient with hemoglobin C trait

Days after Fe^{59}	Hemoglobin fraction	Radioactivity	Ratio of specific activities of components where total = 100
		cpm/mg. Hgb.	
3	Total hemolysate	36	100
	A	30	83
	C	55	153
4	Total hemolysate	57	100
	A	49	86
	C	84	147
5	Total hemolysate	63	100
	A	58	92
	C	100	159
6	Total hemolysate	77	100
	A	66	86
	C	111	144
14	Total hemolysate	87	100
	A	87	100
	C	134	154
18	Total hemolysate	84	100
	A	76	90
	C	122	145
21	Total hemolysate	87	100
	A	81	93
	C	120	138
25	Total hemolysate	86	100
	A	83	97
	C	107	124
69	Total hemolysate	63	100
	A	62	98
	C	61	97

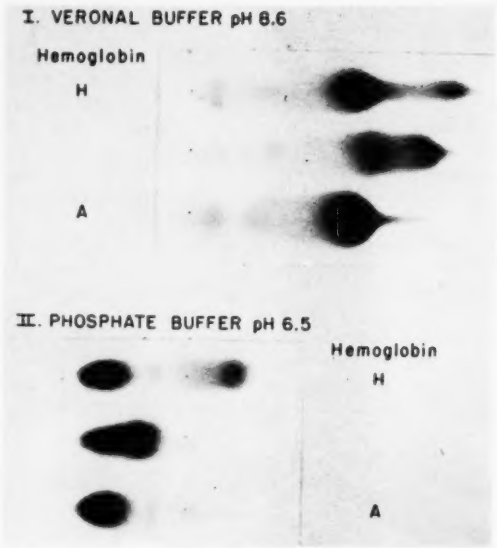

FIG. 1. FILTER PAPER ELECTROPHORETIC PATTERNS IN DIFFERENT BUFFERS OF NORMAL ADULT AND H HEMOGLOBIN TOGETHER WITH THE HEMOGLOBIN OF PATIENT S.P. WHO HAD AN ELECTROPHORETICALLY RAPID ABNORMAL HEMOGLOBIN

This hemoglobin is designated as "J group hemoglobin" (5). Electrophoresis for six hours at 450 volts; paper stained with bromphenol blue.

[1] The difference in radioactivity in the recrystallized hemin of C and A hemoglobins may be within the error of the methods since only a small amount of hemin from hemoglobin C was recovered.

TABLE IV

Studies of Fe^{59} incorporation in hemoglobin components of patient with an electrophoretically abnormal rapid hemoglobin *

Days after Fe^{59}	Hemoglobin fraction	Radioactivity	Ratio of specific activities of components where total = 100
		cpm/mg. Hgb.	
1	Total hemolysate	55	100
	A	50	91
	Rapid component*	53	96
3	Total hemolysate	163	100
	A	150	92
	Rapid component	163	100
6	Total hemolysate	237	100
	A_2	185	78
	A	250	105
	Rapid component	234	99
9	Total hemolysate	239	100
	A	235	98
	Rapid component	242	101
13	Total hemolysate	263	100
	A	252	96
	Rapid component	253	96
16	Total hemolysate	269	100
	A	266	99
	Rapid component	260	97

* This component may have represented hemoglobin J or another rapid component (see text).

ported by the finding of approximately equal Fe^{59} activity in the hemoglobin A and C components isolated from the final blood specimen obtained 69 days after the administration of the isotope and more than three months after the last transfusion. At that time hemoglobin A from previous transfusions had disappeared.

c). Electrophoretically rapid abnormal hemoglobin. Patient S.P. who had an electrophoretically rapid abnormal hemoglobin was an American Negro; approximately 50 per cent of his hemoglobin had an electrophoretic mobility faster than hemoglobin A at pH 8.6. He was presumed to have hemoglobin J on the basis of electrophoretic mobility of hemoglobin specimens in acid and alkaline buffers (see Figure 1) (4), but no family studies could be carried out. Since this abnormal hemoglobin might also have been another electrophoretically rapid abnormal hemoglobin, *e.g.*, hemoglobin N, rather than hemoglobin J, it is designated as "electrophoretically rapid hemoglobin component" or "J group hemoglobin" (5) in this study. The results of the studies of Fe^{59} incorporation in hemoglobin A and the rapid major component are recorded in Table IV. Again no significant difference in Fe^{59} incorporation was noted between hemoglobin A and the major abnormal fraction.

d). Fetal hemoglobin. Patient J.R. had a large amount of fetal hemoglobin in association with multiple myeloma. By the method of alkali denaturation, the fetal hemoglobin amounted to 40 per cent of the total hemoglobin, and similar values were obtained by chromatographic techniques. The patient was known to have had a normal hemoglobin concentration during an unrelated brief

TABLE V

Studies of Fe^{59} incorporation in various hemoglobin components in patients with alkali resistant hemoglobin

Days after Fe^{59}	Hemoglobin fraction*	Radioactivity	Ratio of specific activities of components where total = 100
		cpm/mg. Hgb.	
a. Patient J. R.			
1	Total hemolysate	10.3	100
	Fetal	9.8	95
	Remainder	9.4	91
3	Total hemolysate	101	100
	Fetal	106	105
	Remainder	87	86
4	Total hemolysate	151	100
	Fetal	144	95
	Remainder	142	94
6	Total hemolysate	191	100
	Fetal	178	93
	Remainder	172	90
8	Total hemolysate	203	100
	Fetal	196	96
	Remainder	193	95
13	Total hemolysate	243	100
	Fetal	225	93
	Remainder	205	84
b. Patient L. B.			
5	Total hemolysate	7.3	100
	Fetal	15.2	208
6	Total hemolysate	7.9	100
	Fetal	14.4	182
7	Total hemolysate	7.4	100
	Fetal	15.8	214
13	Total hemolysate	7.0	100
	Fetal	14.4	206
17	Total hemolysate	6.7	100
	Fetal	16.5	246

* See text for methods employed in separation of fetal hemoglobin.

TABLE VI

Studies of Fe^{59} incorporation in hemoglobin components of healthy rabbits

Rabbit I				Rabbit II			
Days after Fe^{59}	Hb fraction	Radio-activity	Ratio of specific activities of components where total = 100	Days after Fe^{59}	Hb fraction	Radio-activity	Ratio of specific activities of components where total = 100
		cpm/mg. Hgb.				*cpm/mg. Hgb.*	
3	Total hemolysate	4,352	100	6	Total hemolysate	3,065	100
	Fast	1,940	45		Fast	1,137	37
	Main	4,692	108		Main	3,002	98
16	Total hemolysate	3,881	100	22	Total hemolysate	2,887	100
	Fast	2,324	60		Fast	2,218	77
	Main	4,198	108		Main	2,945	102
48	Total hemolysate	2,591	100	48	Total hemolysate	2,072	100
	Fast	2,985	115		Fast	1,951	94
	Main	2,547	98		Main	2,040	98
71	Total hemolysate	1,770	100	71	Total hemolysate	1,382	100
	Fast	1,671	94		Fast	1,047	76
	Main	1,553	93		Main	1,400	101

illness three years earlier; he did not have microcytosis and the values for hemoglobin A_2 were normal. Consequently he probably belonged to the group of patients with normal hemoglobin concentration and "hereditary persistence of fetal hemoglobin production" recently summarized by Jacob and Raper (6). In this patient the hemoglobin components were separated chromatographically for the Fe^{59} incorporation studies; the results (see Table V, a) showed the alkali resistant hemoglobin to have the same radioactivity as the remainder of the hemoglobin.

Patient L.B. who had thalassemia major had required whole blood transfusions two to three times monthly for several years. He had been transfused three weeks before Fe^{59} incorporation studies were done. As a result of multiple transfusions, he had extensive hemosiderosis; decreased hemoglobin production and dilution of the isotope by increased storage iron probably accounted for the low levels of radioactivity observed in the hemoglobin. In this patient, the fetal hemoglobin was separated by alkali; the greater isotopic activity of the fetal component (see Table V, b) was attributed to dilution of hemoglobin A by previous transfusion.

2. Minor components of human hemoglobin

On starch electrophoresis, two minor components of hemoglobin, hemoglobin A_2 and hemoglobin A_3, described by Kunkel and Bearn (7), were isolated during studies of Patient O.G. (Table II), and a few observations on hemoglobin A_2 were made during the study of Patient S.P. (Table IV). In Patient O.G. the isotopic activity of the electrophoretically rapidly moving hemoglobin A_3 component was significantly lower than the activity of the major fractions in the early period after the administration of Fe^{59}. However, the relative isotopic activity of the A_3 component appeared to increase somewhat in the later specimens, although Fe^{59} activity never reached the values observed in the principal components. A less striking initial decrease in the isotopic activity of the minor basic component, hemoglobin A_2, was also noted in O.G. and in S.P. Storage of the hemoglobin specimens in the cold and repeating the separations after a period of 10 days did not significantly alter the relative isotopic activities of the components.

3. Rapid hemoglobin component in experimental animals

Preliminary experiments showed that shortly after the administration of Fe^{59}, the "spear" or electrophoretically most rapid component of the hemoglobin of dogs, rabbits and mice exhibited Fe^{59} activity which was significantly lower than the isotopic activity of the main hemoglobin com-

ponent. To test the effect of erythrocyte aging upon the relative activity of the slow component, two rabbits were given, respectively, 40 and 60 μc. of Fe^{59} and hemoglobin separations were carried out at intervals thereafter. The results of the experiments in these rabbits are recorded in Table VI. If the life span of the rabbit erythrocyte is taken to be about 60 days (8, 9), the increase in isotopic activity of the rapid hemoglobin component correlated well with cell aging.

DISCUSSION

Major and minor components of human hemoglobin

Because hemoglobin S and hemoglobin A coexist in most of the erythrocytes of subjects with sickle cell trait, it is reasonable to suppose that both hemoglobins are synthesized simultaneously before maturation of the cell. If this is the case both hemoglobins would be expected to exhibit the same isotopic activity although the total amount of each might show considerable variability in different patients with the trait. In the present study Fe^{59} activity of the hemoglobins of the circulating erythrocytes was measured; this technique probably would not yield evidence distinguishing between simultaneous, alternating or sequential synthesis of different hemoglobins in the immature erythrocytes of the bone marrow. However, the study of Kunkel and Bearn (7) of minor human hemoglobin components and the data on Fe^{59} incorporation into minor hemoglobin components in this study indicate that the interrelationships of various hemoglobins are not necessarily simple since Fe^{59} activity of the hemoglobins may change with time. The fact that minor components exhibit lower isotopic activities than the normal major component has been interpreted as evidence of change in hemoglobin with erythrocyte aging (7), although other explanations are possible. Following the administration of Fe^{59} the minor rapid hemoglobin component, hemoglobin A_3, showed low Fe^{59} activity which subsequently increased; in contrast, in the absence of previous transfusions, the two major components of hemoglobin in patients with sickle cell trait, hemoglobin C trait, alkali resistant hemoglobin, and "group J hemoglobin" trait both showed comparable Fe^{59} activity throughout the period of study. These observations provide some evidence against changes in *major* hemoglobin components with erythrocyte aging.

Studies in patients who had been previously transfused (L.B. and J.B.) demonstrated the effect of previous transfusions in diluting hemoglobin A, with resultant higher isotopic activity in the fetal and hemoglobin C components.

From the data obtained by recrystallization of heme from the hemoglobin components isolated by starch electrophoresis, most of the radioactivity appeared to be due to Fe^{59} in the heme of hemoglobin, although some (nonspecific) adsorption of the isotope elsewhere or incorporation into heme enzymes may have occurred.

In view of the decreased Fe^{59} activity of the fast component (A_3) of normal adult hemoglobin, the data concerning Fe^{59} incorporation in the patient with an electrophoretically rapid *major* component are of particular interest. In that patient, S.P., Fe^{59} incorporation into the electrophoretically rapid hemoglobin did not differ significantly from the incorporation into hemoglobin A_1. However, lower radioactivity of small amounts of hemoglobin A_3 might not be detected in the presence of the large amount of rapid hemoglobin. The findings of the present study concerning major components of hemoglobin are in agreement with those of Motulsky who found no significant differences in Fe^{59} incorporation in major hemoglobin components in patients with hemoglobin H-thalassemia and sickle cell-hemoglobin C disease (10).

The diminished radioactivity of the electrophoretically rapid (A_3) component of human hemoglobin, when compared with the isotopic activity of the lower main (A_1) component, was first observed by Kunkel and Bearn (7); these investigators also noted that this difference appeared to be related to erythrocyte aging. In the present study, a gradual increase with time in the Fe^{59} activity of the electrophoretically rapid A_3 component was again noted. Precise correlation of this effect with erythrocyte aging was not possible because of the probable occurrence of accelerated erythrocyte destruction in patients with disseminated neoplastic disease. With accelerated erythrocyte destruction, particularly of the random variety, reincorporation of Fe^{59} into newly synthesized hemoglobin might result in consistently

higher isotopic activity in the A_1 hemoglobin component.

Electrophoretically rapid hemoglobin component in experimental animals

Studies of Fe^{59} incorporation into the electrophoretically rapid component of hemoglobin in two normal rabbits yielded data which could be better correlated with erythrocyte aging than the data obtained in man. In these rabbits a gradual increase in the isotopic activity of the fast component was noted during the first 48 days of the study. The decline which was observed at 70 days in the relative Fe^{59} activity of the most rapid hemoglobin component in one of these animals may have been related to reincorporation of the isotopic iron into the newly formed main hemoglobin component.

These observations in experimental animals indicate that an electrophoretically rapid hemoglobin may be formed in aging erythrocytes as suggested by Kunkel and Bearn (7). However, our preliminary studies of young and old erythrocytes separated by osmotic hemolysis (11, 12) have failed to demonstrate differences in relative isotopic activity between the hemoglobin components in the young resistant cells as contrasted with the older more fragile cells. Furthermore, the hypothesis that erythrocyte aging is responsible for the formation of this electrophoretically rapid component fails to explain the appearance of significant isotopic activity in the rapid component within a few days after the administration of Fe^{59} when Fe^{59} labeled hemoglobin should be present only in young red blood cells.

Further studies of this electrophoretically rapid hemoglobin component may disclose a correlation with decreasing enzyme levels in aging erythrocytes. However, the influence of factors other than cell aging (for example, hemoglobin distribution within the erythrocyte) on the formation of this electrophoretically rapid hemoglobin component should also be studied.

SUMMARY

1. The incorporation of Fe^{59} into the major components of hemoglobin in individuals with sickle cell trait, hemoglobin C trait, electrophoretically rapid major hemoglobin component of the "J group," probable hereditary persistence of fetal hemoglobin production and thalassemia major revealed no difference in Fe^{59} incorporation in the major components.

2. Observations on the minor electrophoretically rapid components of hemoglobin in a patient with sickle cell trait confirmed the observations of Kunkel and Bearn (7) that the isotopic activity of this hemoglobin A_3 component gradually increased during the 50 day period of study.

3. The studies of the incorporation of Fe^{59} into the electrophoretically rapid component of the hemoglobin of rabbits also supported the hypothesis that the formation of an electrophoretically rapid hemoglobin component in experimental animals is correlated with erythrocyte aging. The possibility that factors other than cell aging may influence the formation of this electrophoretically rapid normal component was discussed.

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OBSERVATIONS ON THE CONCENTRATING AND DILUTING MECHANISMS OF THE DISEASED KIDNEY *

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The concentrating and diluting mechanisms of the normal kidney contribute in an impressive manner to the defense of water economy. In health, a given amount of solute may be excreted in a widely varying volume of water which is determined under most circumstances by the water needs of the organism. This ability to regulate the osmolality of the urine accounts for a range of urine concentrations which extends from marked hypotonicity to marked hypertonicity.[1] In the course of advancing renal disease, the total amount of solute excretion per 24 hours may remain comparable to values achieved in health. It is a clinical observation of long standing, however, that the patient's ability to vary the volume of water in which this solute is contained becomes progressively limited. Hence, the range over which urine tonicity may be extended becomes more and more restricted. Typically, this restriction is manifested initially by a decrease in the maximum attainable urine osmolality, followed somewhat later by a progressive inability to decrease the tonicity of the urine. Ultimately, urine tonicity deviates little in either direction from the concurrent plasma osmolality, and the stage of so-called "permanent isosthenuria" or "fixed specific gravity" supervenes.

The intrarenal mechanisms responsible for the impaired ability to concentrate and dilute the urine in chronic renal disease have never been adequately explained. Although the opinion is widely held that the specific tubular sites for the concentrating and diluting operations are destroyed by the underlying pathologic processes, no experimental confirmation of this thesis has yet appeared. On the contrary, certain theoretic considerations render this explanation open to serious question. Thus, within the context of the conventional theory, the loss of concentrating ability before that of diluting ability must be related to pathologic involvement of the site (or sites) of concentration in the tubule prior to the site of dilution. Moreover, this sequence of events must be identical in all chronic renal diseases associated with the classic pattern of functional alterations, irrespective of the etiology or pathologic characteristics of the specific disease entity. The supposition that such an orderly and predictable progression of anatomic alterations may occur in a heterogeneous group of renal diseases is difficult to reconcile with recorded histologic data. Within recent years several groups have favored an alternative explanation (2–5). This would attribute the impaired ability to concentrate and dilute the urine in chronic Bright's disease to *functional* adaptations in the persisting nephrons of the diseased kidney.[2] Refractory isosthenuria could, therefore, evolve in the face of normal concentrating and diluting mechanisms in the surviving nephrons.

Evidence has recently been presented from this laboratory which indicates that the persisting nephrons in the chronically diseased kidney retain many normal functional characteristics (6). The present studies represent an attempt to investigate the functional integrity of the concen-

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[1] A discussion of current concepts of the concentrating and diluting mechanisms of the normal kidney may be found in a recent paper by Berliner, Levinsky, Davidson and Eden (1).

[2] The two adaptations which have been considered are: *1*) a continuing osmotic diuresis due to high filtered loads of urea; and/or *2*) an increased glomerular filtration rate per functioning nephron. Either of these could account for high rates of urine flow past the concentrating and diluting sites.

trating and diluting mechanism in the diseased kidney. An experimental model has been constructed which allows for the study of a diseased kidney in an essentially normal internal environment. Three different forms of renal disease have been induced experimentally in dogs. In each animal, the lesion has been produced in only one kidney and the opposite organ has been maintained intact. The control kidney serves as a standard of comparison for the diseased organ. Moreover, the normal kidney prevents significant abnormalities from occurring in body fluids, regardless of the severity of the contralateral renal lesion. If the development of impaired concentrating and diluting ability in a diseased kidney results from disruption of tubular transport systems, refractory isosthenuria should ultimately occur regardless of either the functional capacity of the opposite kidney, or the environment in which the diseased organ resides. Conversely, if the progressive inability to modify the tonicity of the urine depends upon adaptive changes (*i.e.*, continuing osmotic diuresis) in functionally intact nephrons, the diseased kidney should retain essentially normal concentrating and diluting characteristics in an environment which minimizes any such adaptations invoked by the uremic state.

METHODS

Sixty experiments were performed on 17 female mongrel dogs. In each animal one of three types of renal disease was induced in a single kidney and the opposite organ was maintained intact. The forms of unilateral renal disease include the following: *1*) A chronic disease which is characterized by marked contraction of the involved kidney and histologic abnormalities of the persisting nephrons including dilatation and atrophy of tubules, intratubular casts, interstitial fibrosis, decrease in size of some glomerular tufts and thickening of glomerular basement membranes. This lesion which has been previously described in detail (*7*) is produced by perfusing the totally anoxic kidney with a solution of an aminonucleoside (6-dimethyl aminopurine 3-amino-D ribose) and diverting the renal venous return to prevent recirculation. *2*) Unilateral pyelonephritis produced by exposing one kidney through a flank incision, massaging it manually for five minutes, puncturing the renal parenchyma approximately 150 times with a 22 gauge needle and injecting 1 ml. of a four hour culture of *E. coli* intravenously. Following the injection of bacteria the ureter is clamped for 20 minutes (*8*). Histologically, the diseased kidneys demonstrate changes of both acute and chronic pyelonephritis. *3*) A reversible renal lesion induced by rendering the experimental kidney anoxic for 30 minutes and then perfusing it with a saline solution under moderate pressure. The histologic changes are limited to the tubules and include vacuolated epithelial cells and intratubular casts (*7*).

The concentrating capacity of the diseased kidney was studied, employing three different protocols. The maximum urine/plasma osmolal ratio was determined following water withdrawal for at least 14 hours. The maximum value for solute-free water abstracted ($T^c_{H_2O}$) was measured during osmotic diuresis induced by mannitol and with constant vasopressin infusion. Values for $T^c_{H_2O}$ were also recorded during the infusion of hypertonic saline. *The diluting capacity* of the diseased kidney was investigated following the intragastric administration of from 40 to 70 ml. per Kg. of water containing 5 ml. of absolute alcohol. Additional alcohol was infused in 0.45 per cent saline during the course of each experiment. The diluting capacity was also studied during isotonic expansion of extracellular fluid volume. In a number of experiments, a moderate amount of water (20 to 30 ml. per Kg.) was administered without alcohol in order to study intermediate ranges of water diuresis.

In each animal a preliminary bladder-splitting procedure was performed in order to construct two permanent hemi-bladders. Each of the latter drains one kidney and allows for evaluation of the separate functions of the individual kidneys. Clearance studies were performed with the animals in the standing position and unanesthetized state. Creatinine and PAH were routinely included in the sustaining infusions. Details of the clearance techniques and analytic procedures have been described in previous communications (*7*, *9*). In all experiments, the behavior of the diseased kidney was contrasted with the simultaneous pattern of the contralateral intact organ.

RESULTS

Concentrating capacity

During osmotic diuresis and with maximum vasopressin activity, the concentrating mechanism of the normal kidney in the dog abstracts from 5 to 8 ml. of water per 100 ml. of glomerular filtrate. This volume of water reabsorbed in excess of an isosmotic equivalent of solute is referred to as $T^c_{H_2O}$. Measurement of $T^c_{H_2O}$ has been made in 20 experiments on 11 dogs (six with an aminonucleoside-induced lesion and five with pyelonephritis) during mannitol and Pitressin® (vasopressin) infusion. Two representative experiments, one on an animal with an aminonucleoside-induced lesion and the other with pyelonephritis, are shown in Table I. Glomerular filtration rates of the experimental (diseased) kid-

TABLE I

Concentrating capacity of the diseased and intact kidneys during the infusion of mannitol and vasopressin in two animals with unilateral renal disease *

Clearance period	GFR		V		Cosm		Posm	$T^c_{H_2O}$		$T^c_{H_2O}$/GFR ×100	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.		Exp.	Cont.	Exp.	Cont.
	ml./min.		*ml./min.*		*ml./min.*		*mOsm./L.*	*ml./min.*			
A. Dog Pr. (14.8 Kg.) Aminonucleoside-induced lesion (185 days after induction)											
1	11.2	39.4	2.0	4.7	2.62	7.14	301	0.66	2.49	5.9	6.3
2	11.2	43.6	2.4	6.2	3.15	9.0	302	0.76	2.85	6.8	6.5
3	11.5	40.9	2.5	5.9	3.29	8.77	303	0.77	2.85	6.7	7.0
4	12.1	43.0	2.7	6.8	3.53	10.10	306	0.84	3.30	6.9	7.8
5	12.1	43.0	2.7	6.6	3.49	9.88	309	0.81	3.27	6.7	7.6
B. Dog Te. (7.5 Kg.) Pyelonephritis (29 days after induction)											
1	14.7	23.5	4.7	5.4	4.84	6.03	322	0.19	0.68	1.3	2.9
2	15.1	23.5	4.7	5.9	5.0	6.56	333	0.28	0.66	1.8	2.8
3	15.6	23.7	4.5	5.9	5.02	6.83	335	0.52	0.98	3.3	4.1
4	13.6	23.3	3.9	5.4	4.57	6.71	337	0.67	1.33	4.9	5.7
5	14.1	23.0	3.8	5.3	4.55	6.72	346	0.73	1.47	5.2	6.4

* Exp. refers to experimental (diseased) kidney; Cont. refers to control (intact) kidney; V, urine flow; Cosm, osmolal clearance; $T^c_{H_2O}$, solute-free water abstracted in the concentration process; Posm, plasma osmolality.

neys were significantly decreased both as compared with the original level of function before induction of disease and with the concurrent value of the control (intact) kidney. Values for $T^c_{H_2O}$ for the experimental kidneys were also appreciably below those for the control kidneys. However, in both dogs, the ratios of solute-free water abstracted per 100 ml. of glomerular filtrate ($T^c_{H_2O}$/GFR × 100) were well within the normal range. Moreover, $T^c_{H_2O}$/GFR for the experimental kidney closely approximated the values for the intact organ. The slight superiority of values for the intact kidneys has been noted in 17 of the 20 experiments and will be referred to again.

In Table II, the concentrating capacity of the diseased organ in an animal with an aminonucleoside-induced lesion is shown during the administration of 5 per cent sodium chloride. The pattern is identical to that seen during the mannitol and vasopressin experiments. Thus, although GFR in the diseased kidney was decreased by over 60 per cent, the values for $T^c_{H_2O}$/GFR were within the normal range, and closely approximated those for the intact organ.

The maximum urine/plasma osmolal ratios for the diseased and intact kidneys are shown in Table III for several representative dogs. In all animals, the experimental organ has retained the ability to elaborate a hypertonic urine, although the values have typically been somewhat less than those for the control organs.

Diluting capacity

Representative experiments for two dogs subjected to water-loading and alcohol administration

TABLE II

Concentrating capacity of the diseased and intact kidneys during the infusion of 5 per cent NaCl at 5 ml. per minute in a dog with unilateral aminonucleoside-induced renal disease *

Clearance period	GFR		V		Cosm		Posm	$T^c_{H_2O}$		$T^c_{H_2O}$/GFR ×100	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.		Exp.	Cont.	Exp.	Cont.
	ml./min.		*ml./min.*		*ml./min.*		*mOsm./L.*	*ml./min.*			
1	16.0	46.5	1.17	2.61	1.92	5.01	336	0.75	2.40	4.7	5.2
2	17.9	45.6	1.30	2.69	2.14	5.17	347	0.84	2.48	4.7	5.4
3	17.1	48.8	1.40	3.03	2.24	5.73	353	0.84	2.70	4.9	5.5
4	15.7	43.3	1.45	2.93	2.27	5.41	362	0.82	2.48	5.2	5.7
5	17.1	44.3	1.56	3.27	2.38	5.86	373	0.82	2.59	4.8	5.9

* Dog He. (10.1 Kg.). The experiment was performed 51 days after induction of the lesion.

TABLE III

Maximum osmolal urine/plasma ratios of diseased and intact kidneys

Dog	Wt.	Day*	GFR Exp.	GFR Cont.	Posm†	Uosm† Exp.	Uosm† Cont.	Max. U/P ratio† Exp.	Max. U/P ratio† Cont.
	Kg.		*ml./min.*		*mOsm./L.*	*mOsm./L.*			
He.‡	10.2	35	11.7	33.3	293	675	1,250	2.30	4.27
Pr.‡	15.2	49	11.9	41.2	289	1,139	1,413	3.94	4.89
Sp.§	9.5	17	16.7	22.3	297	945	1,092	3.18	3.68
Te.§	7.5	29	14.7	23.5	300	1,097	1,391	3.66	4.64
Fu.§	12.7	35	18.3	28.5	294	881	1,002	3.0	3.41

* Day refers to the time elapsed after induction of the lesion.
† Posm, plasma osmolality; Uosm, urine osmolality; Max. U/P ratio, maximum urine/plasma osmolal ratio.
‡ Aminonucleoside-induced disease.
§ Pyelonephritis.

are shown in Table IV. One dog (IV-A) had an aminonucleoside-induced lesion; the other (IV-B) had chronic pyelonephritis. Expression of data is similar to that employed in the concentration studies except that free-water clearance (C_{H_2O}) rather than $T^c_{H_2O}$ has been measured. The term $C_{H_2O}/GFR \times 100$ indicates the number of ml. of free-water in the urine per 100 ml. of glomerular filtrate. Despite marked reduction in GFR, values for C_{H_2O}/GFR were within the normal range for the diseased kidneys. Of some interest, however, is the unexpected and quite consistent finding that C_{H_2O}/GFR was greater for the diseased organs than for the intact kidneys.

The diluting capacity of the diseased kidney was also studied during isotonic expansion of extracellular fluid volume. The results of such an experiment performed on an animal with an aminonucleoside-induced lesion are shown in Table V. Although GFR for the experimental kidney was less than 5 ml. per minute C_{H_2O}/GFR increased progressively and, as was noted during water-loading and alcohol administration, the values for this ratio exceeded those for the intact organ.

Twenty-five experiments have been performed on 10 dogs following the administration of a moderate dose of water (*i.e.*, 20 to 30 ml. per Kg.) without alcohol. These studies permit the comparison of the diseased and intact kidneys during the ascending limb of a sub-maximal water diuresis. The results of experiments on four dogs are shown in Table VI. Two animals had a reversible lesion induced by saline perfusion of the anoxic kidney; one had an aminonucleoside-induced lesion and one had pyelonephritis. In the

TABLE IV

Diluting capacity of the diseased and intact kidneys following water-loading and alcohol administration

Clearance period	GFR Exp.	GFR Cont.	V Exp.	V Cont.	Cosm Exp.	Cosm Cont.	Posm	C_{H_2O}* Exp.	C_{H_2O}* Cont.	$C_{H_2O}/GFR \times 100$ Exp.	$C_{H_2O}/GFR \times 100$ Cont.
	ml./min.		*ml./min.*		*ml./min.*		*mOsm./L.*	*ml./min.*			
A. Dog Pr. (15.2 Kg.)	Aminonucleoside-induced lesion (52 days after induction)										
1	13.9	47.8	1.84	4.78	0.62	1.53	296	1.22	3.25	8.8	6.8
2	14.2	48.5	1.99	4.90	0.68	1.52	287	1.31	3.38	9.2	7.0
3	13.2	45.8	2.14	5.20	0.69	1.51	300	1.45	3.69	11.0	8.1
4	14.9	46.4	2.25	5.31	0.70	1.57	305	1.55	3.74	10.4	8.1
5	12.6	44.7	1.82	4.60	0.53	1.35	306	1.29	3.25	10.2	7.3
6	11.9	40.6	1.67	4.26	0.47	1.32	309	1.20	2.94	10.1	7.2
B. Dog Ho. (12.3 Kg.)	Pyelonephritis (57 days after induction)										
1	21.8	40.7	2.85	2.85	1.04	1.26	273	1.81	1.59	8.3	3.9
2	22.6	36.7	3.15	4.01	0.87	1.20	274	2.28	2.81	10.1	7.7
3	19.5	36.8	2.37	3.85	0.57	1.06	276	1.80	2.79	9.2	7.6

* C_{H_2O}, free-water clearance.

TABLE V

Effects of sustained isotonic expansion of extracellular fluid (ECF) volume on diluting capacity of the diseased and intact kidneys *

Clearance period	GFR		V		Cosm		Posm	C_{H_2O}		C_{H_2O}/GFR ×100	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.		Exp.	Cont.	Exp.	Cont.
	ml./min.		*ml./min.*		*ml./min.*		*mOsm./L.*	*ml./min.*			
1	3.7	21.6	0.21	1.15	0.08	0.44	277	0.13	0.71	3.5	3.3
2	4.0	22.0	0.42	2.22	0.25	0.53	288	0.17	1.69	4.3	7.7
3	3.8	22.5	0.64	2.43	0.31	0.66	288	0.33	1.77	8.7	7.9
4	4.9	26.4	0.67	2.86	0.24	0.85	290	0.43	2.10	8.8	7.6
5	5.0	24.1	0.76	2.56	0.32	0.76	288	0.44	1.80	8.8	7.5

* Dog Fr.(8.2 Kg.). Aminonucleoside-induced lesion (109 days after induction). A simulated ECF solution was infused at the rate of 8.8 ml. per minute. Expanded volume was maintained by concurrent infusion of synthetic urine solutions.

majority of experiments, C_{H_2O}/GFR increased in a roughly parallel manner bilaterally. However, values for C_{H_2O}/GFR generally were greater for the experimental kidneys.

DISCUSSION

The present studies were designed to investigate the concentrating and diluting capacities of the diseased kidney. Within recent years several groups have suggested that isosthenuria in chronic bilateral renal disease may develop despite the persisting integrity of the concentrating and diluting mechanisms in the surviving nephrons. This represents a departure from the conventional explanation which holds that the disease processes produce intrinsic abnormalities of the sites of concentration and dilution in the nephron. The experimental model in the present studies may help to distinguish between these two possibilities. Each animal possessed one normal and one diseased kidney. The presence of the normal kidney dictates that the combined (bilateral) nephron population must always exceed 50 per cent of the original number. Because of this, plasma urea levels have remained near-normal, and any compensatory increase in GFR (per remaining neph-

TABLE VI

Diluting capacity of the diseased and intact kidneys following sub-maximal water-load

Clearance period	GFR		V		Cosm		Posm	C_{H_2O}		C_{H_2O}/GFR ×100	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.		Exp.	Cont.	Exp.	Cont.
	ml./min.		*ml./min.*		*ml./min.*		*mOsm./L.*	*ml./min.*			
A. Dog Sl. (14.2 Kg.) Reversible lesion (21 days after induction)											
1	15.5	37.0	1.48	2.11	0.48	1.38	268	1.00	0.73	6.5	2.0
2	13.8	36.7	1.60	2.91	0.40	1.22	273	1.20	1.69	8.7	4.6
3	14.3	35.5	1.58	3.35	0.38	1.11	274	1.20	2.24	8.4	6.3
B. Dog Da. (15.4 Kg.) Reversible lesion (13 days after induction)											
1	21.1	39.2	1.5	2.38	0.59	0.96	280	0.91	1.42	4.3	3.6
2	20.8	36.9	1.52	2.49	0.57	0.89	281	0.95	1.60	4.6	4.3
3	18.5	38.1	1.27	2.58	0.49	0.93	284	0.78	1.65	4.2	4.3
C. Dog Fr. (8.0 Kg.) Aminonucleoside-induced lesion (210 days after induction)											
1	6.5	44.7	0.10	1.25	0.16	0.77	265	−0.06	0.48	−0.9	1.1
2	5.5	38.0	0.24	2.25	0.14	0.78	266	0.10	1.47	1.8	3.9
3	6.8	36.2	0.38	2.37	0.15	0.61	266	0.23	1.76	3.4	4.9
4	6.7	38.8	0.45	2.75	0.14	0.68	267	0.31	2.07	4.7	5.3
D. Dog Go. (17.4 Kg.) Pyelonephritis (25 days after induction)											
1	37.2	43.4	0.73	0.46	0.66	0.67	265	0.07	−0.21	0.2	−0.5
2	33.7	40.6	1.68	1.42	0.65	0.68	267	1.03	0.74	3.1	1.8
3	34.1	39.4	2.13	2.12	0.52	0.57	270	1.61	1.55	4.7	3.9

ron) imposed by a marked decrease in bilateral nephron population would be minimized. If the conventional theory is substantially correct, the destruction of the sites of concentration and dilution should result in impaired concentrating and diluting ability in the diseased kidney regardless of the presence of an intact kidney, or of the status of body fluids. Conversely, if the isosthenuria results from a continuing osmotic diuresis (due either to high filtered loads of urea, or to an increased GFR/functioning nephron), the diseased kidney in the present experimental model might retain essentially normal concentrating and diluting characteristics.

In all animals studied, regardless of the severity of the renal lesion in the experimental kidney, values for the concentrating and diluting capacities (when expressed as ml. of $T^c_{H_2O}$ or C_{H_2O} per 100 ml. of glomerular filtrate) have been within the normal range. These data serve to document the essential integrity of the concentrating and diluting processes in the persisting nephrons of the diseased kidneys.[3] The same data suggest that the permanent isosthenuria of chronic Bright's disease may in most instances be explicable on the basis of functional rather than anatomic changes.

One functional change which occurs in the course of chronic progressing bilateral renal disease would appear to be of fundamental importance in the genesis of the restricted range of urine osmolalities. This consists of the fact that an ever-decreasing number of nephrons continue to excrete an essentially constant amount of solute. In order to accomplish this, each nephron must excrete an increasing fraction of its glomerular filtrate. The concentrating and diluting mechanisms of individual nephrons are thus brought to bear on an ever-increasing volume of tubular urine. Because of the rate limitations of these mechanisms, the degree to which the final urine tonicity may be modified must decrease asymptotically towards isosmolality.

The concentrating mechanism would appear to be impaired before the diluting mechanism for several reasons. First, the absolute change from isosmolality (*e.g.*, 300 mOsm. per L.) is considerably greater for a maximally concentrated urine (*circa* 1,200 mOsm. per L.) than for a maximally dilute urine (*circa* 50 mOsm. per L.). A 50 per cent reduction in range on either side of isosmolality, due to a twofold increase in tubular flow past the active sites, would seem to represent a much greater defect in concentration than dilution. Second, the amount of water abstracted per unit of glomerular filtrate in the concentrating process is approximately half the amount of free-water engendered in the diluting process (11). That a third factor may contribute to the loss of the ability to concentrate the urine before the ability to dilute the urine is suggested by certain observations in the present study. Thus, the data, while demonstrating the relative integrity of the concentrating and diluting mechanisms of the diseased organs, have also revealed certain differences between the diseased and intact kidneys of individual animals. These differences are characterized by *slightly lower* values for $T^c_{H_2O}$/GFR for the experimental kidney, and *slightly higher* values for C_{H_2O}/GFR for the experimental kidney. The urine from the diseased kidney, therefore, always has a greater amount of free-water (either relative or absolute) per unit of filtrate than that of the normal organ. The existence of a similar phenomenon in both kidneys of the patient with bilateral renal disease would contribute further to the masking of the concentrating mechanism before that of the diluting mechanism. Studies are currently in progress to extend these observations.

In Figure 1, a theoretical formulation is presented of the influence of progressive renal disease on the range of urine osmolalities if *the concentrating and diluting capacities of all functioning nephrons remain normal.* It is assumed that at each level of glomerular filtration rate 600 mOsm. of solute is excreted per 24 hours. Each nephron is assumed to be capable of removing 5 ml. of water per 100 ml. of glomerular filtrate in the concentrating process (*i.e.*, $T^c_{H_2O}/GFR \times 100 = 5$), and of engendering 10 ml. of free-water per

[3] Recent studies by Baldwin, Berman, Heinemann and Smith (10) on patients with *bilateral* renal disease are in general agreement with this conclusion. In the majority of subjects, values for $T^c_{H_2O}$/GFR were within the normal range. In those in whom values were below normal, it is not yet apparent whether this may be attributed to an absolute decrease in $T^c_{H_2O}$ or to an increased GFR per nephron.

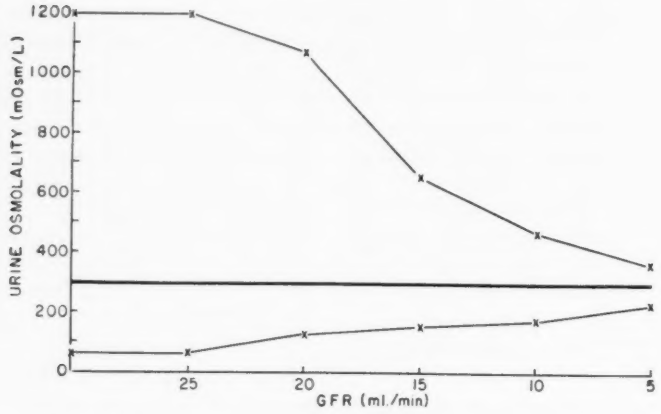

FIG. 1. THEORETICAL CURVES SHOWING MAXIMUM AND MINIMUM OSMOLALITIES IN PROGRESSIVE RENAL INSUFFICIENCY IF *CONCENTRATING AND DILUTING CAPACITIES OF EACH FUNCTIONING NEPHRON REMAIN NORMAL*

At each level of glomerular filtration rate, solute excretion is assumed to equal 600 mOsm. per 24 hours.

100 ml. of filtrate in the diluting process (*i.e.*, $C_{H_2O}/GFR \times 100 = 10$). Below a GFR of 20 ml. per minute progressive inability to concentrate the final urine is noted. Ability to dilute effectively persists longer but eventually diminishes markedly also.

SUMMARY

The mechanisms responsible for the impaired ability to concentrate and dilute the final urine in chronic progressive renal disease have never been adequately explained. The conventional theory holds that the disease process results in anatomic destruction of the concentrating and diluting sites in the nephron. An alternative concept holds that the concentrating and diluting mechanisms of the persisting nephrons remain intact and attributes the ultimate development of permanent isosthenuria to an increasing volume of urine flowing past the sites of these operations. In the present studies, the integrity of the concentrating and diluting mechanisms of the diseased kidney has been investigated in an attempt to distinguish between these two theories. One of three varieties of experimental renal disease has been induced in a single kidney of dogs in which the opposite kidney has been maintained intact as a control. The normal kidney served to prevent the evolution of body fluid abnormalities seen in bilateral renal disease. If the conventional theory is correct, the destruction of the sites of concentration and dilution in the diseased kidney should result in refractory isosthenuria regardless of the status of body fluids or of the presence of a contralateral intact organ.

In all the animals studied, regardless of the severity of the renal disease or the degree of anatomic disorganization, values for the concentrating and diluting capacities (expressed as ml. of $T^c_{H_2O}$ or C_{H_2O} per 100 ml. of glomerular filtrate) of the experimental kidneys were within the normal range. These data serve to document the essential integrity of the concentrating and diluting processes in the persisting nephrons of the diseased kidney, and strongly imply that the pathologic processes do not destroy specific functional sites in the tubule. A theoretical explanation has been offered which allows for the decreasing range of urine osmolalities in bilateral renal disease despite intact concentrating and diluting processes.

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THE EFFECTS OF HEMORRHAGE ON PULMONARY CIRCULATION AND RESPIRATORY GAS EXCHANGE *†

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A close relationship between pulmonary ventilation and blood flow exists in the normal functioning of the lungs. Thus, primary alterations in one of these processes are difficult to distinguish from secondary changes in the other. In order to study these two functions in the intact animal, we have separated them by utilizing a technique which allows us to induce alterations in pulmonary blood flow while keeping ventilation constant in rate and tidal volume. This report describes changes observed in the pulmonary circulation and respiratory gas exchange of dogs in whom blood flow was decreased by hemorrhage and then restored by blood replacement.

Studies on experimental animals and human subjects have indicated that all pulmonary vessels are not open at the same time (1), and that the pulmonary circulation is able to accommodate a marked increase in blood flow with little rise in the pulmonary arterial blood pressure (2–7). An increase in blood flow, then, must be accompanied by a decrease in vascular resistance, presumably due to expansion of the pulmonary bed. However, the mechanisms by which such accommodation occurs have not been fully elucidated, and questions remain as to the relative importance of physical forces, the effectiveness of vasomotor control and the significance of local pressor and chemo-reflexes. Moreover, only fragmentary data are available regarding the effects of primary *reduction* in the pulmonary blood flow, as in hemorrhagic shock (8–10), or after diversion of blood around the lungs, as in open heart surgery.

The results of the present study contribute information on the response of the pulmonary vessels to a reduction in blood flow. Our findings suggest that as flow is diminished portions of the pulmonary vascular bed close completely, and that with restoration of flow there is some delay before these vessels reopen.

METHODS

Eight dogs, ranging in weight from 12 to 19 Kg. (mean, 15 Kg.), were used in these experiments. The animals were anesthetized with sodium pentobarbital (35 mg. per Kg.) and infused with succinylcholine (0.4 μg. per ml.) at a rate sufficient to arrest respiratory muscular activity; no supplemental anesthesia was administered.

a). Respiratory measurements. The animals were intubated with a cuffed endotracheal tube and ventilated with room air by a specially designed respirator, which intermittently delivered a known tidal volume at a constant flow rate ("square wave") and of uniform duration (11). Expiration was passive with the airway open to the atmosphere. The expired gas was passed through a dry gas flow meter in which it was thoroughly mixed; at selected intervals samples were collected and analyzed for oxygen and carbon dioxide concentrations (12). In addition, a small stream of gas from the endotracheal tube was constantly drawn through an infrared carbon dioxide gas analyzer[1] so that a continuous record of the end-tidal carbon dioxide partial pressure could be obtained (13, 14).

At the outset of each experiment the respiratory frequency and tidal volume were adjusted so as to maintain the end-tidal gas carbon dioxide partial pressure within the normal range; once set, the ventilation was kept constant throughout the course of each study.

The endotracheal pressure was continuously measured, so that a pressure-volume curve for each inflation could be obtained. Since the tidal volume remained constant, the slope of this curve (Figure 1) directly reflects the total lung-thorax compliance as the change in intrapulmonic volume (tidal volume) per unit change in transthoracic (endotracheal) pressure (15–17).

[1] Liston-Becker, Model 16.

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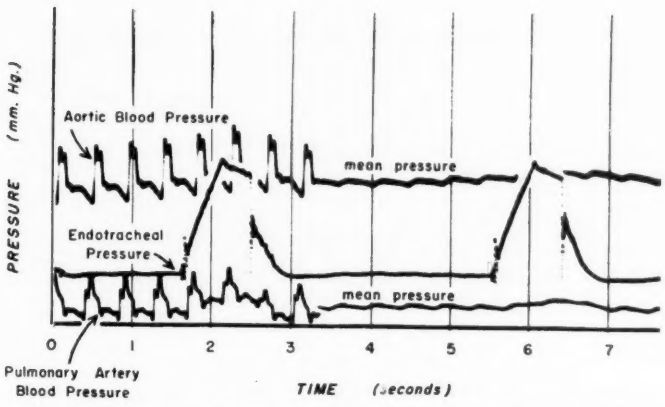

FIG. 1. SEGMENT OF CONTINUOUS RECORD OF INSTANTANEOUS AND ELECTRICALLY INTEGRATED MEAN SYSTEMIC AND PULMONARY ARTERIAL BLOOD PRESSURES AND ENDOTRACHEAL AIR PRESSURE

b). *Blood pressure measurements.* A cardiac catheter was passed via the external jugular vein and positioned, under fluoroscopic guidance, so that its tip rested in the main pulmonary artery. Another catheter was placed in one femoral artery. Using P23D Statham strain gages, we obtained simultaneous tracings of the pulmonary and femoral arterial blood pressures on a cathode ray oscilloscope; these were photographically recorded (Figure 1).

c). *Blood samples.* At appropriate intervals during each experiment, blood samples totaling 15 ml. [approximately 1 per cent of the estimated blood volume (18)] were obtained simultaneously from the femoral and pulmonary arteries and analyzed as follows:

1). Oxygen saturation was determined spectrophotometrically using the Beckman D.U. spectrophotometer and the Nahas cuvette, according to the method of Holling, MacDonald, O'Halloran and Venner (19).

2). Oxygen and carbon dioxide contents were determined by the micromanometric method of Natelson as modified by Holaday and Verosky (20).

3). pH was determined by passing blood directly from the catheters in the femoral and pulmonary arteries through a glass electrode pH meter, as described by Holaday (21).

4). Hematocrit was measured using the microcentrifuge technique (22).

5). Lactic acid concentration of arterial blood was determined by the method of Barker and Summerson (23).

d). *Calculations.* From the simultaneously obtained blood and gas samples we calculated the following:

1). Carbon dioxide tension of arterial and mixed venous blood from the Singer-Hastings nomogram (24) using the values obtained for the blood carbon dioxide content, pH and hematocrit.

2). Pulmonary blood flow by the direct Fick principle using the values for the blood and gas oxygen contents.

3). Respiratory (physiological) dead space by the Bohr formula using the carbon dioxide values and substituting the arterial for the alveolar carbon dioxide tensions (25). Thus:

$$\text{respiratory dead space} = \frac{(Pa_{CO_2} - P_{ECO_2})\,V_T}{Pa_{CO_2}}$$

where Pa_{CO_2} and P_{ECO_2} are the carbon dioxide tensions (mm. Hg) in the arterial blood and mixed expired gas, respectively, and V_T is the tidal volume. When used in this manner, the total respiratory (physiological) dead space is equivalent to that portion of the tidal volume which does not undergo gas exchange with pulmonary capillary blood (26).

4). Percentage of venous admixture from the blood oxygen contents (ml. O_2 per 100 ml. blood), using the ratio of the concentration differences of arterial to end-pulmonary capillary blood:mixed venous to end-pulmonary capillary blood, as: $(Cc'_{O_2} - Ca_{O_2})/(Cc'_{O_2} - C\bar{v}_{O_2})$ (27); "Ca_{O_2}" and "$C\bar{v}_{O_2}$" refer to the oxygen concentrations in the arterial and mixed venous bloods, respectively, and "Cc'_{O_2}" refers to the "ideal" end-pulmonary capillary blood which is assumed to be fully saturated.

5). Buffer base of arterial and mixed venous blood from the Singer-Hastings nomogram, using the values obtained for carbon dioxide content, oxygen saturation, pH and hematocrit (24).

e). *Estimates of errors in the methods.* All analyses were performed in duplicate. The reproducibility (one standard deviation) of the analytical methods was as follows:

1). Blood analyses: oxygen saturation, ±1 per cent; oxygen content, ±0.2 volume per cent; carbon dioxide content, ±0.1 mM per L.; pH, ±0.007; hematocrit, ±1.0 per cent; lactic acid, ±10 μg. per ml.; buffer base, ±0.5 mEq. per L.

2). Gas analyses: oxygen and carbon dioxide concentrations, ±0.04 per cent; carbon dioxide partial pressure, ±0.5 mm. Hg.

f). *Estimates of variance.* The above data are contained in Tables I through VI. Wherever possible the standard deviation $\left(\text{S.D.} = \sqrt{\frac{\Sigma\,(\bar{x} - x)^2}{n - 1}}\right)$ and the standard error

TABLE I

Relative changes in mean systemic and pulmonary arterial blood pressures and lung-thorax compliance during hemorrhage

Estimated blood volume reduction (%)	Control	7	12	17	22	27	32	37	42	48
Number of dogs	8	8	8	8	8	6	5	3	2	1
Mean femoral arterial blood pressure (% of control)	100*	89	78	68	52	45	39	42	33	30
(Range)		(70–98)	(60–93)	(42–90)	(30–80)	(25–72)	(75–65)	(30–60)	(20–47)	
± 1 standard deviation		9.6	13.4	15.6	17.9	17.2	15.4			
Standard error of mean		3.4	4.8	5.5	6.3	7.0	6.8			
Mean pulmonary arterial blood pressure (% of control)	100*	93	85	78	73	67	65	68	65	65
(Range)		(90–100)	(72–100)	(70–93)	(65–90)	(60–83)	(55–82)	(55–80)	(55–75)	
± 1 standard deviation		7.8	10.8	9.4	9.2	10.6	11.4			
Standard error of mean		2.8	3.8	3.3	3.2	4.3	5.1			
Lung-thorax compliance (% of control)	100*	99	98.0	97.5	96.0	94.5	90.0	89.5	88.5	85.0
(Range)		(97–100)	(95–100)	(92–100)	(88–100)	(92–97)	(88–95)	(87–92)	(87–90)	
± 1 standard deviation		1.26	2.43	2.48	4.80	1.70	3.32			
Standard error of mean		0.44	0.86	0.91	1.70	0.69	1.48			

* The actual mean values during the control period were: 135 mm. Hg for the femoral arterial blood pressure, 13.0 mm. Hg for the pulmonary arterial blood pressure, and 30.5 ml. per mm. Hg for the lung-thorax compliance.

of the mean $\left(\text{S.E.}_m = \frac{\text{S.D.}}{\sqrt{n}}\right)$ are given ("$\bar{x}$" represents the mean, "x" the individual variation and "n" the number of determinations). In those cases where only the mean values and the range are given, we intend to show merely the direction of change, the degree of which was proportional to the percentage of the blood volume removed which varied from animal to animal.

g). Procedure. After a 30 minute equilibration period two sets of control blood and gas samples were obtained one hour apart. We then began to bleed the animals from the other femoral artery in successive steps, each approximating 5 per cent of the assumed blood volume [estimated as 10 per cent of the body weight (18)], until the systemic blood pressure fell to between 25 and 50 per cent of the control level. After each aliquot of blood was removed, three minutes were allowed for re-equilibration. A sustained hypotension was obtained after approximately one-third of the estimated blood volume had been removed.

Over the next hour we collected three more sets of blood and gas samples, after which all the blood was replaced in similar step-wise increments.[2] Another two sets of data were obtained during the hour following reinfusion. We then sacrificed the animals by injecting concentrated potassium chloride into the left ventricle. As soon as the heart beat ceased, the chest was rapidly opened and the hilum of one lung clamped to prevent leakage of air and blood. The lungs were removed, fixed in formalin and microscopic sections prepared.

[2] Three of the eight animals died before complete data could be obtained for the postreinfusion period.

RESULTS

A. *Systemic and pulmonary arterial blood pressure values*

Figure 2 and Tables I and II portray the relative changes in the mean systemic and pulmonary arterial blood pressures and total lung-thorax compliance in terms of the percentage of their control values. The data plotted are the averaged values of the mean pressures obtained three minutes following completion of each successive step in the process of bleeding and reinfusion.

During blood volume depletion the systemic and pulmonary arterial blood pressures both decreased until the pulmonary pressure reached approximately two-thirds of its control value (from 13 to 8.5 mm. Hg). Despite additional blood

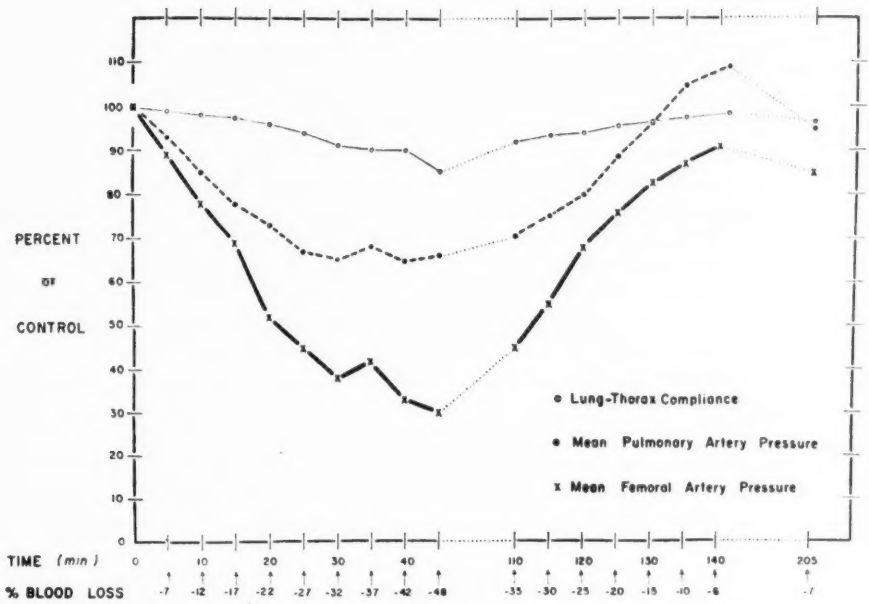

FIG. 2. RELATIVE CHANGES IN AVERAGE MEAN SYSTEMIC AND PULMONARY ARTERIAL BLOOD PRESSURES AND TOTAL LUNG-THORAX COMPLIANCE DURING HEMORRHAGE AND DURING BLOOD REPLACEMENT

Values for the control period are: main pulmonary artery blood pressure, 13 mm. Hg; femoral artery blood pressure, 135 mm. Hg; lung-thorax compliance, 30.5 ml. per mm. Hg.

loss, the pulmonary pressure then stabilized at this level, falling only slightly, whereas the systemic pressure continued its marked decline. When the blood was replaced, the systemic pressure returned to only 90 per cent of its prehemorrhagic level, whereas the pulmonary pressure *exceeded* its control value by 10 per cent.

B. *Blood flow*

During the control period the blood flow averaged 2.6 L. per minute. Following blood removal, the flow fell to between 21 to 35 per cent of the control value. After the blood was reinfused the blood flow returned to 68 per cent of the control level (Table III, Figure 3).

C. *Respiratory dead space*

The total respiratory (physiological) dead space, as derived by the above methods, is the sum of the "anatomical" or airway dead space, and the so-called "alveolar" dead space (28–34). The "anatomical" portion of the dead space is a virtual volume equivalent to the volume of the respiratory passages leading to the alveoli. The "alveolar" dead space represents the volume of air ventilating alveoli which are either not perfused, or inadequately perfused, and depends upon the overall ventilation-perfusion relationship in the alveoli throughout the lung (26).

Under normal conditions, the ventilated alveoli are well perfused so that the "alveolar" dead space is negligible and the total respiratory (physiological) dead space is equivalent to the "anatomical" dead space alone (31, 32). When, however, blood flow is diminished in relation to ventilation, a significant "alveolar" dead space may develop in addition to the "anatomical" dead space (26, 34).

During the control period the total dead space comprised 33 per cent of the tidal volume (dead space, 74 ml.; tidal volume, 220 ml.) (Table III, Figure 3). There was no significant arterial to end-tidal carbon dioxide tension difference (Table

IV, Figure 4), so that we can assume that no significant "alveolar" dead space existed, all the dead space being "anatomical." Following removal of one-third of the estimated blood volume, the total respiratory (physiological) dead space increased to 55 per cent of the same tidal volume (120 ml.), and an arterial to end-tidal carbon dioxide tension gradient appeared which averaged 8 mm. Hg. This indicates the development of a significant "alveolar" dead space. When the blood volume was restored (minus the 6 per cent expended for samples) the dead space volume returned toward the control volume (90 ml.) and the carbon dioxide tension difference decreased to 2.5 mm. Hg. The increase in the mixed venous blood carbon dioxide tension presumably reflects only the decreased cardiac output; it returned to its control level after blood restoration (Tables III and IV, Figures 3 and 4.)

The percentage of venous admixture was reduced, following hemorrhage, from a control value of 18 to 11 per cent. After blood volume was restored, this was further decreased to 5 per cent, and then rose to 7 per cent one hour after blood volume restoration (Table III).

During the control period the average oxygen saturation of the arterial blood was 95 per cent and of the mixed venous blood, 72 per cent. Following hemorrhage, the mixed venous blood oxygen saturation decreased to only 30 per cent, and the arterial blood achieved 90 per cent saturation (Table V, Figure 4). This fall in the arterial oxygen saturation is commensurate with the degree of admixture of markedly unsaturated venous blood and the decreased effective alveolar ventilation. There is thus no evidence of a barrier to gas diffusion; with blood replacement both values rapidly returned to their control levels.

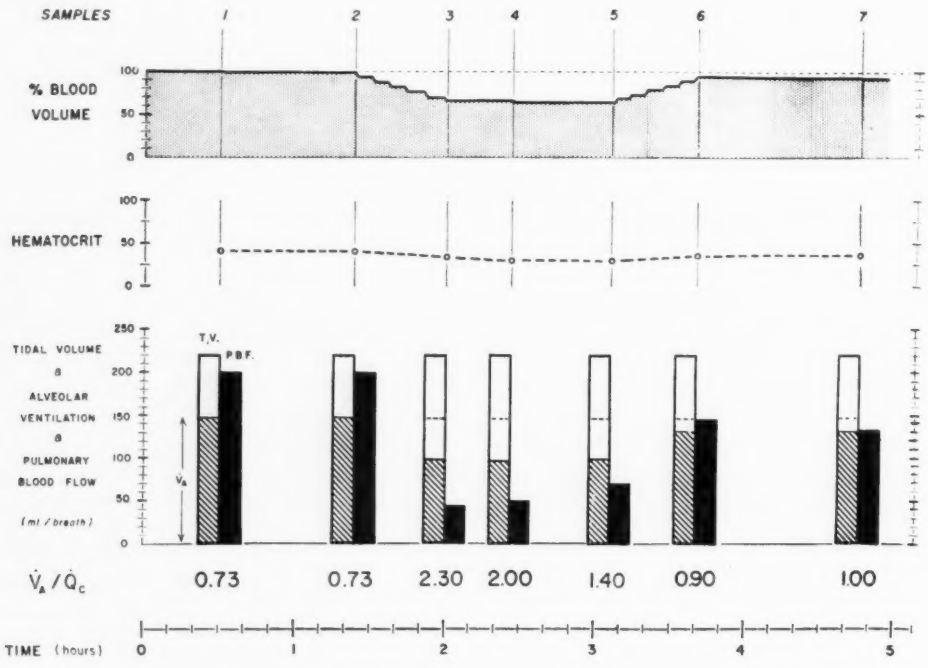

FIG. 3. CHANGES IN PULMONARY BLOOD FLOW PER INFLATION, Q_c (BLACK COLUMNS), AND TIDAL VOLUME, TV, DURING CONTROL PERIOD (1, 2), FOLLOWING HEMORRHAGE (3, 4, 5) AND AFTER BLOOD RESTORATION (6, 7)

The tidal volume is divided into alveolar ventilation, V_A (hatched area), and total respiratory dead space (white area). The dead space is further divided by the broken line into "anatomical" dead space above, and "alveolar" dead space below.

TABLE II

Relative changes in mean systemic and pulmonary arterial blood pressures and lung-thorax compliance during reinfusion

Estimated blood volume reduction (%)	35	30	25	20	15	10	6	7*
Number of dogs	3	5	5	5	5	5	5	5
Mean femoral arterial blood pressure (% of control)	41	55	68	76	83	87	91	85
(Range)	(25–65)	(30–75)	(35–85)	(45–95)	(55–100)	(60–100)	(65–100)	(55–100)
± 1 standard deviation	19.0	17.0	19.8	19.7	18.9	15.8	14.7	19.7
Standard error of mean	11.0	7.0	8.8	8.8	8.5	7.1	6.6	8.8
Mean pulmonary arterial blood pressure (% of control)	71	74	80	88	96	105	110	95
(Range)	(60–90)	(60–90)	(65–100)	(70–110)	(80–120)	(85–125)	(90–130)	(65–120)
± 1 standard deviation	16.1	13.2	14.5	14.5	14.1	15.4	16.9	22.2
Standard error of mean	9.3	5.9	6.5	6.5	6.3	6.9	7.6	9.9
Lung-thorax compliance (% of control)	92.0	94.0	95.0	95.5	96.5	98.0	99.0	98.0
(Range)	(88–95)	(90–98)	(90–100)	(91–100)	(93–100)	(93–100)	(95–100)	(93–100)
± 1 standard deviation	2.85	3.46	4.80	4.58	3.00	2.92	2.23	3.10
Standard error of mean	1.65	1.55	2.15	2.10	1.34	1.31	1.00	1.52

* One hour after all the blood was returned.

The arterial and mixed venous blood pH values declined after hemorrhage, as did the values for the buffer base concentration (Table V, Figure 5). These changes were accompanied by a simultaneous rise in lactic acid concentration, suggesting that products of anaerobic metabolism were the source of the metabolic acidosis which developed.

The total lung-thorax compliance declined to 90 per cent of its control level with blood removal and then returned to the control value following blood replacement (Tables I and II, Figure 2). This decrease could mean either that the lungs became "stiffer" as pulmonary blood flow was reduced, possibly owing to increased bronchomotor tone or changes in pulmonary blood volume, or that there was a decrease in the functioning lung tissue owing to collapse of alveoli (35–38). Histological sections of the sealed lungs from dogs that died during hypotension showed areas of collapse, particularly in the hilar regions (Figure 6), which would tend to support the latter explanation.

DISCUSSION

The findings obtained in this study are significant in two respects. First, with reduction in blood flow the systemic arterial blood pressure decreased continuously, whereas the blood pressure in the main pulmonary artery leveled off after an initial decline. Second, there was a marked increase in the total respiratory (physiological) dead space attributable to the development of a significant "alveolar" dead space. Following the restoration of blood volume, the systemic blood pressure did not return completely to its pre-hemorrhagic level, whereas the pulmonary pressure actually *exceeded* its control value. The total dead space decreased towards the control volume, but remained somewhat greater than before hemorrhage, and the lung-thorax compliance returned to its starting level.

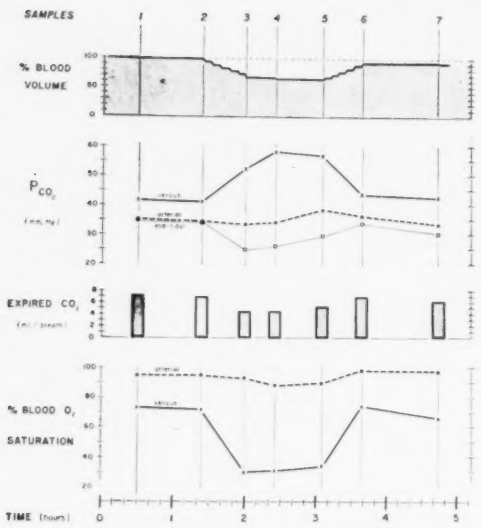

FIG. 4. VARIATIONS IN ARTERIAL AND MIXED VENOUS BLOOD AND END-TIDAL GAS CARBON DIOXIDE TENSIONS, VOLUME OF CARBON DIOXIDE EXPIRED PER VENTILATION, AND ARTERIAL AND MIXED VENOUS BLOOD OXYGEN SATURATIONS DURING THE CONTROL PERIOD (1, 2), FOLLOWING HEMORRHAGE (3, 4, 5) AND AFTER BLOOD RESTORATION (6, 7)

Note the arterial to end-tidal carbon dioxide tension gradient which developed after hemorrhage.

In the face of an equivalent reduction in blood flow, maintenance of the central pulmonary arterial blood pressure, despite a continuous decline of the systemic arterial blood pressure, suggests either an elevation of left auricular pressure or an increase in the pulmonary vascular resistance. To evaluate the first possibility we measured left auricular pressure by direct needle puncture in two additional dogs and found a progressive *decrease* during hemorrhage (Table VI). Thus, it must be assumed that as blood flow decreases the pulmonary vascular resistance rises more rapidly than the systemic resistance. This could occur as a result of: *a*) uniform and progressive vasoconstriction throughout the lungs, or *b*) complete closure of an ever-increasing number of pulmonary vascular channels, or both processes occurring simultaneously. An "alveolar" dead space could develop with either alternative. A distinction between these two mechanisms can be made from our data by examining the blood and gas carbon dioxide tensions, and the blood oxygen saturations.

Normally, there is adequate perfusion of the ventilated alveoli so that an equilibrium for a readily diffusible gas, such as carbon dioxide, will exist between alveolar gas when sampled as end-tidal gas, and end-pulmonary capillary blood when sampled as arterial blood (25, 27, 39–41). We found this to be true for our control determinations. Figure 7 is a schematic portrayal of the lung showing the relationship between pulmonary circulation and ventilation, according to the values obtained from the blood and gas analyses during the *control* period.

Following blood volume depletion, if the blood flow to the ventilated alveoli were reduced *uniformly* throughout the lung, as by partial vasoconstriction, a carbon dioxide equilibrium between end-tidal gas and arterial blood would still exist because of the great diffusibility of this gas (42–44). Our findings, however, show that the end-tidal gas carbon dioxide tension fell below that of the arterial blood (Table IV, Figure 4), so that a marked arterial to end-tidal carbon

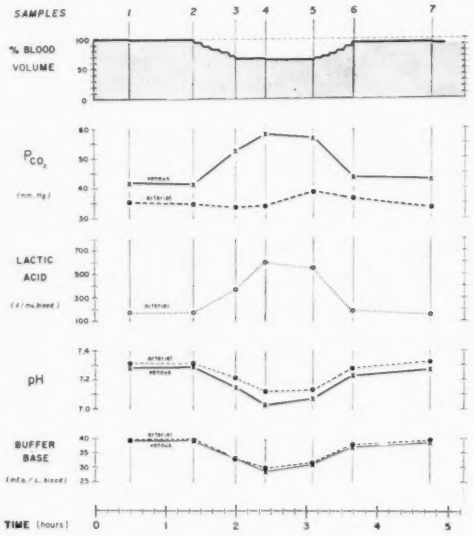

FIG. 5. CHANGES IN ARTERIAL AND MIXED VENOUS BLOOD CARBON DIOXIDE TENSIONS, pH VALUES, AND LACTIC ACID AND BUFFER BASE CONCENTRATIONS DURING CONTROL PERIOD (1, 2), FOLLOWING HEMORRHAGE (3, 4, 5) AND AFTER BLOOD RESTORATION (6, 7)

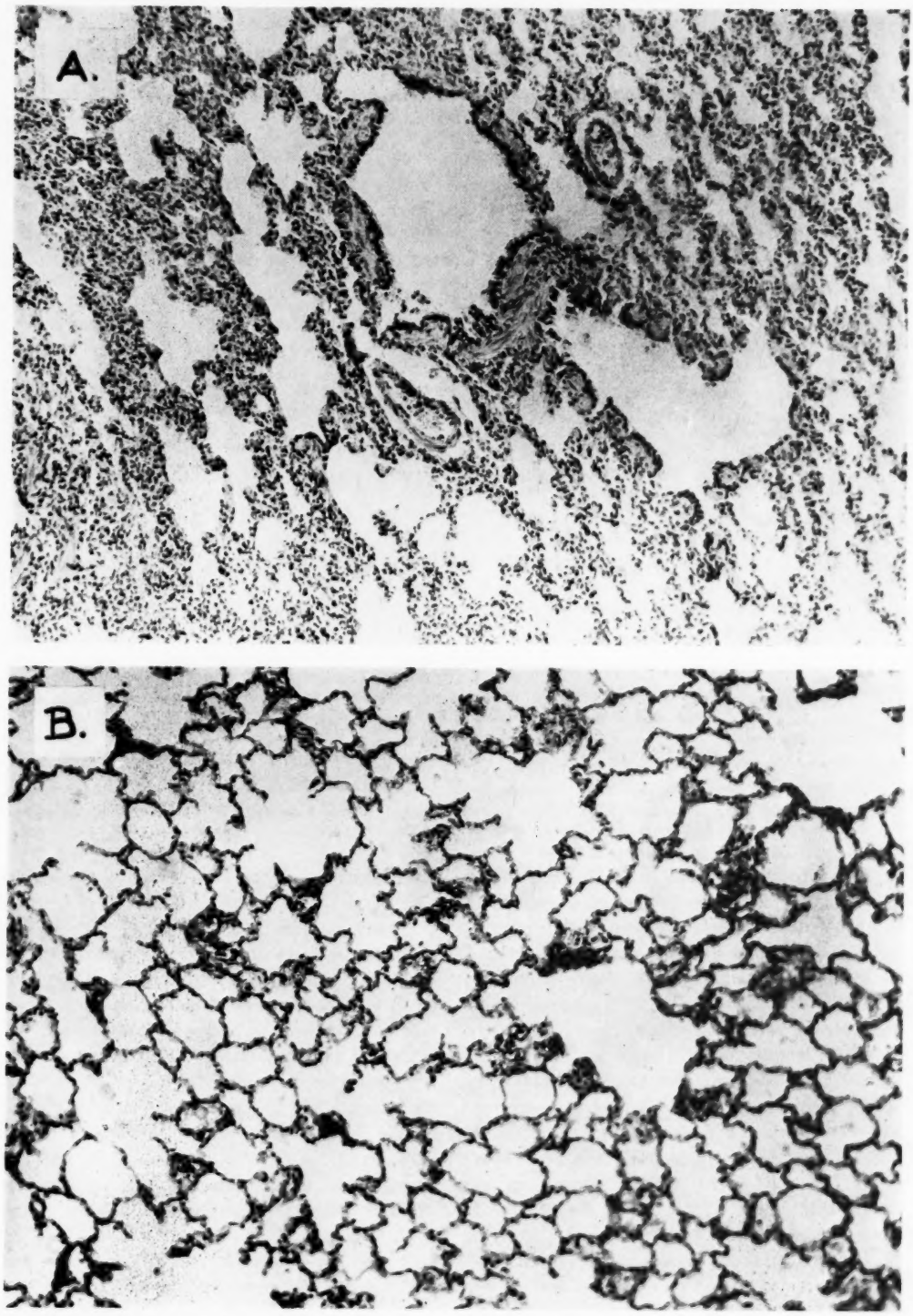

FIG. 6. MICROSCOPIC APPEARANCE OF LUNG SECTIONS FROM A DOG THAT DIED DURING HYPOTENSION

A. Hilar portion showing collapse of alveoli. B. Peripheral portion showing air filled alveoli and relatively avascular parenchyma.

TABLE III

Respiratory and circulatory observations during hemorrhage and reinfusion

	Control		Hemorrhage			Restoration	
Sample	1	2	3	4	5	6	7
Number of dogs	8	8	8	8	8	5	5
Tidal volume (ml.)	220	220	220	220	220	224	224
(Range)	(200–270)	(200–270)	(200–270)	(200–270)	(200–270)	(200–270)	(200–270)
Ventilations per minute	13	13	13	13	13	12	12
(Range)	(11–15.5)	(11–15.5)	(11–15.5)	(11–15.5)	(11–15.5)	(11–13)	(11–13)
Total (physiological) respiratory dead space (ml.)*	74	75	122	120	124	90	88
(Range)	(61–87)	(66–88)	(98–144)	(98–145)	(98–150)	(76–107)	(78–100)
% of control	100	100	164	162	167	120	118
Effective alveolar ventilation (ml. per inflation)*	146	145	98	100	96	134	136
(Range)	(115–206)	(115–204)	(60–168)	(55–167)	(54–163)	(80–163)	(78–177)
% of control	100	100	67	69	66	94	93
Pulmonary blood flow (L./min.)*	2.6	2.6	0.55	0.65	0.92	1.75	1.60
(Range)	(1.1–8.1)	(1.1–5.7)	(0.19–1.1)	(0.25–1.6)	(0.31–2.8)	(1.1–2.4)	(0.63–2.2)
ml. per inflation	200	200	42	50	71	146	134
% of control	100	100	21	25	35	68	62
Estimated blood volume (% of control)*	100	100	67 (52–79)	66 (51–78)	65 (50–77)	94	93
Venous admixture* (% of cardiac output)	16	18	9	11	12	5	7
Ventilation/perfusion ratio*	0.73	0.73	2.30	2.00	1.40	0.90	1.00
Respiratory exchange ratio*	0.90	0.94	1.10	1.10	1.15	0.97	0.90

* The mean value for the entire group is given to indicate the direction of change. The degree of change was proportional to the amount of blood removed, which varied from animal to animal.

dioxide tension difference was present. This indicates that the development of a significant "alveolar" dead space is responsible for the increase in the total (physiological) dead space. Thus, under conditions of reduced pulmonary blood flow we must view the lung as though portions of the pulmonary bed close completely, leading to *nonuniform* perfusion as indicated in Figure 8. Those ventilated alveoli which are no longer perfused cease to take part in gas exchange, and thus contain only that carbon dioxide washed into them from the dead space during inflation (34). On expiration, the gas coming from these alveoli dilutes the carbon dioxide coming from the perfused alveoli, lowering the end-tidal carbon dioxide tension and leading to the development of a carbon dioxide tension gradient between the end-tidal air and the arterial blood. Such a concept is compatible with our findings.

A probable mechanism by which such nonuniform closure of blood vessels could occur involves the relationship between the forces which keep blood vessels open and those which tend to close them. The primary force which distends the vessels is the intravascular blood pressure. This is opposed by the tension within the vessel walls, the surrounding tissue pressure and, in the case of the lung during intermittent positive pressure ventilation, the intrapulmonic air pressure which tends to compress the delicate pulmonary capillaries suspended in an air matrix with little solid support.

As the blood flow decreases there occurs, initially, a fall in the central pulmonary arterial blood pressure. When the blood pressure drops to a

TABLE IV

Arterial and mixed venous blood and end-tidal gas carbon dioxide tensions, and end-tidal to arterial carbon dioxide tension gradients

	Control		Hemorrhage			Restoration	
Sample	1	2	3	4	5	6	7
Number of dogs	8	8	8	8	8	5	5
%[1]Estimated blood volume removed	1	2	33	34	35	6	7
(Range)			(21–48)	(22–49)	(23–50)		
Mixed venous blood P_{CO_2} (mm. Hg)*	41.5	41.0	52.3	57.5	56.5	45.3	42.5
(Range)	(35–56)	(30–57)	(34–78)	(34–91)	(39–87)	(39–56)	(30–57)
Arterial blood P_{CO_2} (mm. Hg)*	35.5	35.5	33.6	34.0	36.0	36.5	33.5
(Range)	(27.9–43.0)	(26.7–42.0)	(23.9–48.3)	(19.5–47.5)	(18.4–44.8)	(27.6–44.1)	(22.3–42.0)
End-tidal gas P_{CO_2} (mm. Hg)*	35.2	34.9	25.3	26.0	28.2	34.0	31.0
(Range)	(29.0–39.0)	(29.5–40.5)	(18.0–32.5)	(15.5–38.0)	(14.0–36.0)	(30.0–39.0)	(21.0–38.0)
Average end-tidal to arterial P_{CO_2} gradient (mm. Hg)*	0.3	0.6	8.3	8.0	7.8	2.5	2.5
(Range)	(−2.3–4.0)	(−2.8–2.9)	(4.2–18.7)	(2.6–21.0)	(1.6–26.7)	(−2.4–6.1)	(−0.4–5.0)

* The mean value for the entire group is given to indicate the direction of change. The degree of change was proportional to the amount of blood removed which varied from animal to animal.

TABLE V

Changes in blood values during hemorrhage and reinfusion

	Control		Hemorrhage			Restoration	
Sample	1	2	3	4	5	6	7
Number of dogs	8	8	8	8	8	5	5
Percentage blood oxygen saturation*							
Arterial	94.7	94.8	93.0	90.0	90.0	98.0	97.0
(Range)	(90.2–97.9)	(89.0–99.1)	(75.8–100)	(70.0–100)	(72.7–100)	(96.1–99.7)	(95.9–98.6)
Venous	72.9	71.7	30.2	32.0	31.2	74.1	66.0
(Range)	(48.1–84.2)	(50–87.2)	(7.2–61.5)	(10.0–66.3)	(11.0–76.7)	(69.2–81.1)	(61.0–71.9)
Hematocrit*							
Arterial	40.3	40.7	34.0	31.0	30.5	35.8	36.9
(Range)	(34.4–48.2)	(34.6–47.8)	(26.9–42.7)	(20.4–41.3)	(24.0–37.0)	(24.3–41.8)	(30.0–42.0)
Venous	41.0	41.5	33.3	31.2	30.8	35.1	37.2
(Range)	(34.2–51.8)	(34.6–49.0)	(27.6–41.5)	(24.5–37.0)	(24.5–37.0)	(30.0–38.6)	(29.1–45.0)
Blood pH*							
Arterial	7.315	7.318	7.212	7.140	7.135	7.285	7.322
(Range)	(7.233–7.388)	(7.218–7.411)	(6.921–7.388)	(6.699–7.388)	(6.700–7.361)	(7.230–7.331)	(7.272–7.402)
Venous	7.281	7.286	7.146	7.053	7.068	7.228	7.270
(Range)	(7.152–7.361)	(7.184–7.390)	(6.827–7.361)	(6.582–7.363)	(6.630–7.314)	(7.168–7.311)	(7.168–7.358)
Blood buffer base (mEq./L.)*							
Arterial	39.6	39.6	33.1	29.6	31.5	37.9	39.3
(Range)	(36.3–44.8)	(36.2–42.8)	(21.8–39.6)	(15.6–40.0)	(15.5–40.2)	(34.2–42.0)	(37.0–42.2)
Venous	39.3	39.4	33.3	28.4	31.0	37.0	38.5
(Range)	(36.0–44.3)	(36.9–42.8)	(20.7–40.6)	(14.2–40.7)	(14.6–40.1)	(33.1–42.3)	(36.4–42.1)
Lactic acid concentration (μg./ml.)*							
Arterial	190	172	380	677	557	182	155
(Range)	(76–338)	(75–257)	(125–965)	(117–1,347)	(165–1,570)	(140–240)	(99–199)

* The mean value for the entire group is given to indicate direction of change. The degree of change was proportional to the amount of blood removed which varied from animal to animal.

TABLE VI

Average mean systemic and pulmonary arterial blood pressures and left atrial pressure in two dogs during hemorrhage and reinfusion

	Control	During hemorrhage								During reinfusion									
Estimated blood volume reduction (%)	0	5	10	15	20	25	30	35	40	40*	35	30	25	20	15	10	5	0	0†
Femoral arterial blood pressure (mm. Hg)	140	135	130	120	115	105	90	70	40	50	75	100	115	120	125	130	135	138	135
Pulmonary arterial blood pressure (mm. Hg)	11.5	10.8	9.5	9.0	8.5	8.0	7.5	7.0	6.5	7.5	9.8	13.0	12.5	13.1	15.3	15.7	17.2	18.0	16.5
Left atrial pressure (mm. Hg)	4.0	3.0	2.5	1.5	1.0	0.5	0	0	0	0	0.5	1.5	2.5	3.5	4.5	5.0	5.5	6.0	5.5

* Measured after a 10 minute interval.
† Ten minutes after all the blood was returned.

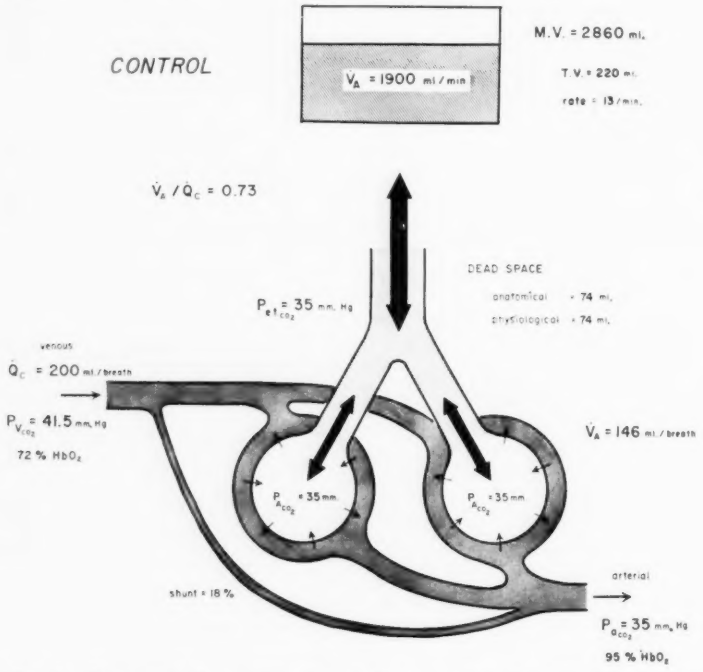

FIG. 7. SCHEMATIC PORTRAYAL OF THE RELATIONSHIP BETWEEN PULMONARY CIRCULATION AND VENTILATION ACCORDING TO THE VALUES OBTAINED DURING THE *CONTROL* PERIOD

The alveoli are represented by the circular areas into which are directed shaded tubes designating conducting airway or "anatomical" dead space. The darkly shaded channels surrounding the alveoli represent pulmonary blood flow. The large arrows entering the alveoli indicate the distribution of the inspired air, and the small arrows represent diffusion of respiratory gases across the alveolar capillary walls. The rectangular block above designates the minute volume, which is divided into total respiratory dead space (white area) and effective alveolar ventilation (hatched area) (26).

$\dot{Q}_c$ = pulmonary blood flow (per inflation), $_A$ = alveolar ventilation, TV = tidal volume, and P_v, P_a, P_A and $P_{e.t.}$ represent the carbon dioxide tensions in mixed venous and arterial bloods, and alveolar and end-tidal gases, respectively. The figures shown are the mean values.

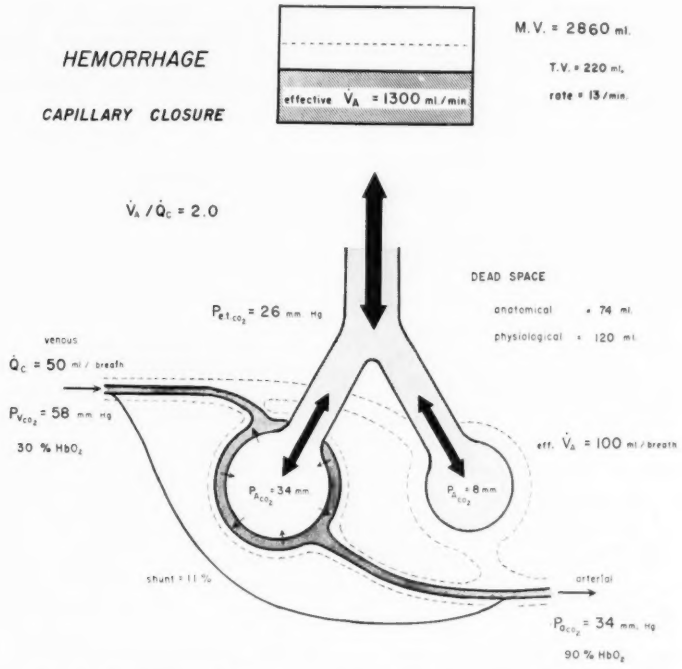

FIG. 8. SCHEMATIC PORTRAYAL OF THE RELATIONSHIP BETWEEN PULMONARY CIRCULATION AND VENTILATION, BASED UPON DATA OBTAINED DURING THE MID-POINT OF THE *HYPOTENSIVE* PERIOD (POINT 4)

The lung is portrayed as if some portions of the pulmonary vascular bed were closed completely, all the "alveolar" dead space being limited to the nonperfused alveoli. In the block representing the minute volume the dead space ventilation, indicated by the white area, is divided by the broken line into "anatomical" dead space above, and "alveolar" dead space below.

critical level below the opposing pressures, some of the pulmonary capillaries may then close completely (45–52). This would evoke a reduction in the size of the vascular bed leading to a rise in vascular resistance; the central pulmonary arterial blood pressure could then be maintained despite a progressive reduction in blood flow. Since these pressure relationships may not be uniform throughout the lung, this process may take place in scattered portions of the lung and lead to closure of vessels despite a blood pressure in the main pulmonary artery considered adequate for perfusion. When the blood flow again increases, the resistance offered by the closed vessels to passive reopening would initially require a driving force in excess of that necessary to maintain flow in patent channels. This force must be provided by increased work on the part of the right ventricle resulting in a rise in the pulmonary arterial blood pressure (Figure 2) (53), or the cardiac output could not be maintained at its control level (54).

We feel that the evidence presented here supports the concept that decrease in pulmonary blood flow can lead to complete closure of pulmonary vessels (55, 56), at least during intermittent positive pressure ventilation. Such a mechanism may be responsible for some of the phenomena ascribed to pulmonary vasomotor activity (57), and may, in part, account for the variations in pulmonary diffusing capacity noted under different circumstances (42, 44, 58–60). It may also help to explain some of the respiratory difficulties occasionally seen during hemorrhagic shock in patients receiving artificial ventilation, or

following complete circulatory bypassing of the lungs, as in open heart surgery (61).

SUMMARY

1. Blood flow in eight anesthetized dogs was decreased by removal of one-third of the estimated blood volume and then restored by blood replacement.

2. The pulmonary and systemic arterial blood pressures, total lung-thorax compliance and end-tidal carbon dioxide gas tension were continuously measured. Pulmonary and systemic arterial blood samples were analyzed for oxygen and carbon dioxide contents, pH, hematocrit values and lactic acid concentrations. Samples of mixed expired air, obtained simultaneously with the blood samples, were analyzed for carbon dioxide and oxygen concentrations. From these data we calculated pulmonary blood flow, respiratory dead space, venous admixture, carbon dioxide tensions and buffer base.

3. The significant findings were as follows:

a. With reduction in blood flow the systemic arterial blood pressure declined continuously, whereas the pulmonary arterial blood pressure stabilized after an initial decline, thereafter dropping only slightly.

b. The respiratory dead space increased following hemorrhage, with the appearance of a marked carbon dioxide tension gradient between the arterial blood and the end-tidal gas, indicating development of a significant "alveolar" dead space.

c. Upon restoration of blood volume the pulmonary blood pressure initially rose above its control level. The respiratory dead space decreased toward its control volume and the carbon dioxide tension gradient was reduced.

4. We conclude that, with intermittent positive pressure ventilation, reduction of pulmonary blood flow may lead to complete closure of portions of the pulmonary bed and that, following restoration of blood flow, there is some delay before these vessels again reopen.

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LOCALIZATION AND RETENTION OF I^{131} FROM FED TRIOLEIN IN THE ATHEROSCLEROTIC INFILTRATION OF RABBIT AORTAS *

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Until recently, many clinical and the laboratory investigators considered an atherosclerotic artery to be a relatively inert tissue and its excess lipid and cholesterol concentration the passive, chronic result of intramural deposit. The demonstration by Siperstein, Chaikoff and Chernick (1), by Eisley and Pritham (2) and Werthessen, Nyman, Holman and Strong (3) of the ability of the aorta of some animals to synthesize cholesterol and the discovery by Biggs and associates (4) of the presence of recently fed cholesterol in human atherosclerotic artery suggested the participation of such an artery in the general day to day cholesterol metabolism of the mammal. Azarnoff has found (5) that aortae of humans, dogs, rats and cats synthesize not cholesterol itself, but another unidentified digitonin-precipitable sterol. Zilversmit, Shore and Ackerman (6) showed not only the existence of amazingly active phospholipid synthesis on the part of the aorta of an atherosclerotic rabbit but also demonstrated a differential activity in such synthesis between an atherosclerotic plaque and neighboring normal areas.

However, the triglyceride dynamics of the atherosclerotic artery are not well known. The present report describes some features of the participation of an atherosclerotic vessel in the disposition of an orally administered triglyceride tagged with I^{131}.

METHODS

Seven rabbits were fed a commercial pellet diet enriched with cholesterol (2 per cent) and cotton seed oil (2 per cent) for three months, after which time they were placed again on ordinary Purina rabbit chow for another three months. Then each of these previously hypercholesteremic rabbits was given by stomach tube 2.0 ml. of iodinated triolein containing 0.73 mc. of I^{131} (Raolein®, Abbott [1]) for three successive days. For control purposes, two normal rabbits were given the same dosage of I^{131} triolein. For additional control purposes, one previously hypercholesteremic rabbit and two normal rabbits were given 2.0 ml. of NaI solution containing 0.73 mc. of NaI^{131} for three successive days. All rabbits were given KI (2 mg. of KI per Gm. of Purina rabbit chow) for two days prior to the feeding of radioactive iodine in order to prevent significant absorption of I^{131} by the thyroid gland.

Seven days after the initial administration of the radioactive triolein or sodium iodide all of the animals were sacrificed. The aorta of each of the previously hypercholesteremic animals was examined and its degree of atherosclerosis was grossly estimated. The atheromata consisted of smooth white glistening thickened areas without fibrosis or lesions either grossly or histologically. Microscopic sections stained with Sudan IV showed the presence of both intra- and extracellular lipid. Then a section of the aorta beginning from the attachment of the semilunar valves was so cut from each rabbit that the total area of intima always equaled approximately 550 sq. mm. This first section was used for determination of total radioactivity. A second section of aorta also was obtained for radioautography. The adventitial fat of these aortic sections was removed with meticulous care. The segments then were weighed. Then, in addition, a section of adrenal, adventitial fat of the aorta, perirenal fat, liver, kidney and thyroid were obtained, weighed, cut up into small fragments and thoroughly washed.

The aorta of each of the normal rabbits given either triolein I^{131} or NaI^{131} was similarly sectioned, cleaned of adventitial fat and weighed.

The I^{131} content of the organ and samples was determined as follows: Organ fragments were placed in uni-

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[1] This material is made by iodinating commercial triolein with iodine monochloride in the proportion of one atom of iodine to 1,000 molecules of triolein. The resulting iodinated fat is then mixed with peanut oil to increase volume in order to facilitate handling. While more than 90 per cent of the I^{131} is lipid bound, in our hands chromatographic analysis has shown the presence of at least five I^{131} containing lipids.

TABLE 1

Radioactivity of tissues after I^{131} administration

Rabbit	Aorta (550 sq. mm.)			Adrenal			Aortic fat			Renal fat			Liver			Kidney			Thyroid			Atherosclerosis
	*cps**	*wt.*†	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*grade*
Normal rabbit + triolein I^{131}																						
C–11	0.080	145	0.001																			0
C–12	0.084	159	0.001																			0
Average	0.082	152	0.001																			
Normal rabbit + NaI^{131}																						
R–4	366	585	0.63	39	160	0.24	189	520	0.36				159	795	0.20	107	495	0.22	106	730	0.15	0
R–5	291	350	0.83	39	150	0.26							194	715	0.27	254	695	0.37	140	750	0.19	0
Average	328	468	0.73	39	155	0.25	189	520	0.36				177	755	0.24	181	595	0.29	123	740	0.17	
Atherosclerotic rabbits + triolein I^{131}																						
A. Rabbits with marked atherosclerosis																						
2031	3,555	1,205	2.95	4,510	660	6.83	752	640	1.18	264	230	1.15	1,215	2,055	0.59	1,425	1,720	0.83	257	950	0.27	5
2053	2,300	800	2.88	8,520	770	11.06				1,257	425	2.95	1,467	2,030	0.72	550	995	0.55	471	1,055	0.45	6
2023	2,910	995	2.92	2,400	475	5.05	918	300	3.06	785	205	3.83	639	975	0.66	1,020	1,140	0.89	502	730	0.69	6
2026	2,060	590	3.49	2,076	330	6.29	767	365	2.10	852	235	3.63	790	1,030	0.77	981	1,185	0.83	179	610	0.29	4
Average	2,706	898	3.06	4,376	559	7.3	812	435	2.11	790	274	2.89	1,028	1,523	0.69	994	1,260	0.78	352	836	0.43	5
B. Rabbits with minimal atherosclerosis																						
2057	1,125	535	2.10	4,560	410	11.1	2,473	525	4.7	3,830	215	17.8	266	285	0.93	226	425	0.53	223	755	0.30	2
2057‡	442	255	1.73																			0
2054	443	425	1.02	3,685	535	6.9	337	455	0.74	154	135	1.14	402	1,125	0.36	264	1,030	0.26	485	795	0.61	1
Average	666	404	1.62	4,123	473	9.0	1,405	490	2.72	1,992	175	9.47	334	705	0.65	245	728	0.40	354	775	0.45	1
Atherosclerotic rabbits with NaI^{131}																						
2028	146	290	0.5	99	620	0.16	40	205	0.1				139	620	0.22	135	535	0.25	101	835	0.12	

* Counts per second.

† Sample weight in milligrams.

‡ An additional segment of aorta showing less atherosclerosis was obtained and measured in this animal.

form glass test tubes, 0.9 per cent saline added to a constant volume of 2 ml., and all samples counted in a well-type scintillation counter with a spectrometer scaler long enough to ensure counting errors of less than 3 per cent. Counts obtained were corrected for decay to the date of injection.

Dental (no screen) film packs were exposed for two half-lives to the second aorta segments of all rabbits. Tissues were maintained hydrated by wrapping with Saran Wrap® and preserved by refrigeration. In the atherosclerotic rabbits given either triolein I^{131} or NaI^{131}, films also were exposed to a section of adrenal and of aortic adventitial fat.

RESULTS

The data indicated convincingly that I^{131} from orally ingested triolein was capable of entering and remaining in an atherosclerotic plaque. Thus in the four rabbits exhibiting thick and confluent aortic atherosclerosis, the radioactivity of the first section of atherosclerotic aorta on a unit weight basis (see Table I) was as intense as that found either in the aortic adventitial fat or in the perirenal fat but not as great as that observed in the adrenal. It is of interest too that the radioactivity of the aortic sections obtained from the severely atherosclerotic rabbits (see Table I) was four times as great as sections of similar area obtained from less severely atherosclerotic rabbits. On a weight basis, however, the intensity appears to be only about twice as great.

On the other hand, the aortic segments of normal rabbits given I^{131} triolein failed to exhibit significant radioactivity (see Table I). As compared with the radioactivity of aortic segments from atherosclerotic rabbits given triolein I^{131}, relatively little radioactivity was found in the aortic segments of either the normal or the atherosclerotic rabbits given NaI^{131}. Additionally, only minimal excess concentration of I^{131} was found in the fatty tissues of these animals given NaI^{131} in contrast to the marked preferential concentration in such tissues of I^{131} from triolein. Apparently any fatty tissue, including the adrenal gland, appears capable of preferentially receiving and retaining, for a time at least, I^{131} from triolein. This capability is much less manifest in the case of inorganic iodine. Such organs, however, as the liver, the kidney and the previously iodide "blocked" thyroid gland either receive or retain relatively little either of triolein I^{131} or NaI^{131} when assayed four days after the last of three daily doses.

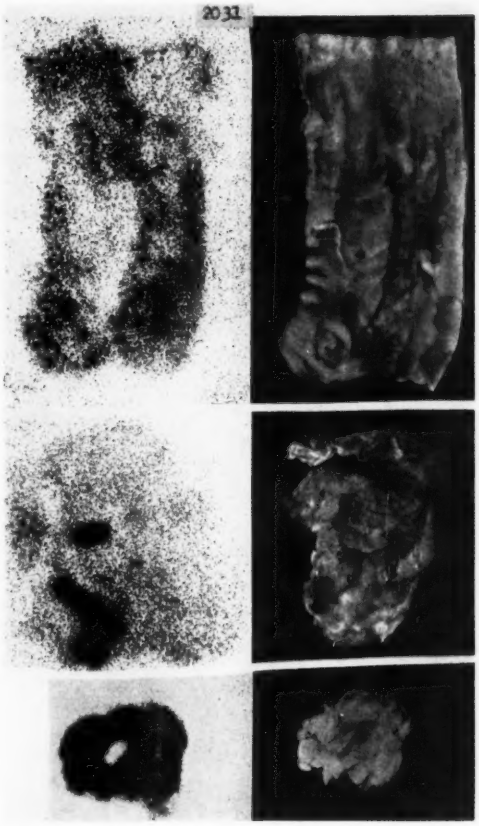

FIG. 1. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT (TOP), AORTIC ADVENTITIAL FAT (MIDDLE) AND MEDIAN CROSS-SECTION OF ADRENAL (BOTTOM) AND THEIR RESPECTIVE RADIOAUTOGRAPHS OF RABBIT 2031 GIVEN I^{131} TRIOLEIN

Note the greater density of the aortic radioautograph in its parts corresponding to the atherosclerotic areas of the aorta itself. Also note the intense density of the adrenal radioautograph as well as the relatively clear zone corresponding to the medullary area of the adrenal.

The radioautographs revealed results quite consonant with those obtained by the well scintillation counter. Thus (see Figure 1) films exposed to sections of the atherosclerotic aorta, aortic fat and adrenal gland from animals receiving triolein I^{131} all revealed radioactivity present therein. Also, as suggested by the results obtained with the counter, the films indicated considerably more

activity in the adrenal gland than in the other fat containing tissues. Moreover, this activity clearly was most intense in the cortex of the adrenal gland. It will be observed (see Figures 1 and 2) that the film of the aortic segments indicates activity primarily from the atherosclerotic plaques and almost none from the normal aortic intima lying between these plaques. As expected, the films (see Figures 3 and 4) exposed to the aortic fragments of the normal rabbit given triolein I^{131} and to the aortic fragments of the atherosclerotic rabbit given NaI^{131}, respectively, revealed no significant radioactivity. It is of interest too that the aortic adventitial fat and adrenal gland of the rabbit given NaI^{131} also failed (see Figure 4) to exhibit significant radioactivity on film.

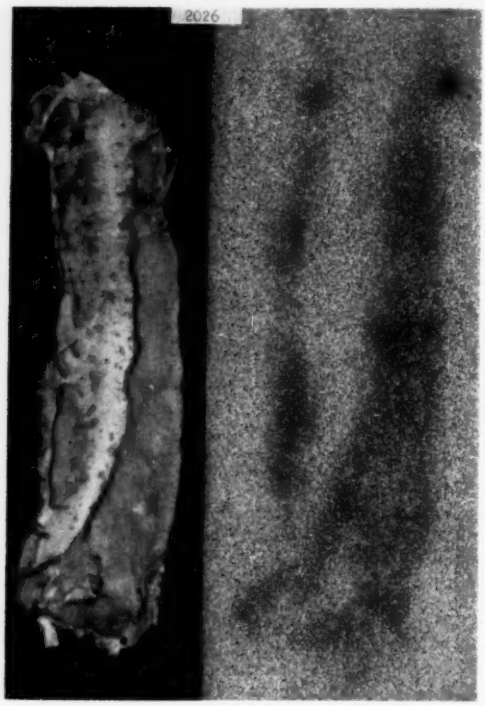

FIG. 2. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT OF RABBIT 2026 GIVEN I^{131} TRIOLEIN (SUDAN STAIN) AND CORRESPONDING RADIOAUTOGRAPH

Note again the correspondence between the darker portions of the radioautograph and the atherosclerotic areas of the aorta stained here with Sudan. The emulsion grain is responsible for the background.

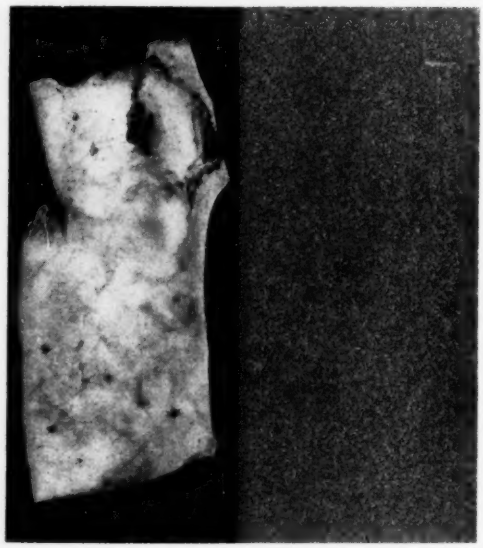

FIG. 3. PHOTOGRAPH OF A LONG SEGMENT OF AORTIC FRAGMENT AND AUTOGRAPH OF NORMAL RABBIT C-11 GIVEN I^{131} TRIOLEIN

Note the background imparted by the emulsion grain and the complete absence of radioactivity as indicated by the autograph.

DISCUSSION

The foregoing results demonstrate decisively that the atherosclerotic aorta of the rabbit is both permeable to and retentive of I^{131} when the latter is administered in lipid form, primarily as triolein I^{131}. Furthermore, the greater radioactivity exhibited by those aortas having the greatest amount of lipid infiltration, the negligible radioactivity of the normal aorta, and finally the localization of the activity afforded by the radioautographs all lead inescapably to the conclusion that it is the atherosclerotic infiltration itself which is responsible for the activity detected.

The ability of the I^{131} to penetrate and to remain in an atherosclerotic process is probably due to its combination with lipid and the probable solubility of this lipid in the lipid of the plaque. This seems true for a number of reasons. It should be recalled that although fed fat is absorbed into the intestinal wall as a mixture of tri-di- and monoglycerides and fatty acids, nevertheless the fat particles in the intestinal lymph contain triglycerides only. Partial glycerides and long chain

fatty acids have disappeared by synthesis into triglyceride during passage across the intestinal wall (*7*). The triglycerides are stabilized as chylomicrons with phospholipid, cholesterol and protein (8) and it is possible for some of the phospholipid to be formed in the intestinal mucosa from dietary constituents (*7*). However, Zilversmit and co-workers have shown (6, 9) that plasma phospholipid is entirely excluded from the normal or atheromatous artery of the rabbit. Hence it is fair to conclude that the I^{131} whose preferential localization in the atheromatous plaque was observed in the present study entered the blood stream in the form of chylomicron triglyceride. Additional reasons for this conclusion are: *1*) relatively little I^{131} was detected in an atherosclerotic infiltration when administered as NaI^{131}, and *2*) other tissues rich in fat, such as adrenal cortex, aortic adventitial and perirenal fat, were also found to localize I^{131} after its administration as triolein I^{131}.

Because seven days were permitted to elapse between feeding I^{131} and killing the animals, it is not possible from these experiments to state whether the atherosclerotic plaque is more or less prone to localize lipid bound I^{131} than the other organs and fatty tissues assayed. Sufficient time has been allowed for considerable metabolic alteration. This was allowed by design, in the hope of obtaining just such radioautographs as were in fact obtained. Only serial studies will determine to what extent the differential localization observed at the end of seven days reflects differences in intrinsic permeability, retention or lipid turnover rate in the tissues studied. Such studies are now under way. Nevertheless, the present experiments indicate that a significant differential localization of I^{131} from triglyceride may take place in a supposedly passive atherosclerotic process.

SUMMARY

Four days after the last of three daily doses of triolein I^{131} was fed to atherosclerotic rabbits, radioactivity was found in the atherosclerotic aorta. Moreover this activity was concentrated preferentially in the atherosclerotic infiltration itself. Radioactivity also was found in other fat containing tissues such as the adrenal cortex, aortic and perirenal fat. In contrast, little activity was found in "non-fatty tissues" such as adrenal medulla, liver, kidney and thyroid. In normal rabbits no radioactivity was found in the aorta after triolein I^{131}.

ACKNOWLEDGMENTS

The authors wish to express their appreciation for the technical assistance of Clarence Omoto and Warren Hayashi, and their gratitude to Dr. Samuel Abraham, Department of Physiology, University of California, for his advice concerning chromatographic procedures.

FIG. 4. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT (TOP), AORTIC ADVENTITIAL FAT (MIDDLE) AND MEDIAN CROSS-SECTION OF ADRENAL (BOTTOM) AND THEIR RESPECTIVE AUTOGRAPHS OF RABBIT 2028 GIVEN RADIOACTIVE IODIDE

Note absence of radioactivity in all tissues including the atherosclerotic aorta.

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THE EFFECT OF CHANGING SERUM OSMOLALITY ON THE RELEASE OF ANTIDIURETIC HORMONE IN CERTAIN PATIENTS WITH DECOMPENSATED CIRRHOSIS OF THE LIVER AND LOW SERUM OSMOLALITY

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Hyponatremia and lowered total solute concentration of the serum are commonly observed in patients with cirrhosis of the liver with ascites and edema (1–4). The mechanisms responsible for this chronic depression of the osmolality of the body fluid are not clear. The normal person maintains the tonicity of his body fluid within narrow limits, and lowering this by the addition of sufficient water to the body promptly results in diminished secretion of antidiuretic hormone (ADH) and the excretion of increased volumes of hypotonic urine until the osmolality of the serum returns to normal (5–7). In contrast, the patient with decompensated cirrhosis may excrete small volumes of hypertonic urine despite a much lower than normal serum osmolality (1–4). However, further lowering of the osmotic pressure in such a patient by the ingestion or infusion of water often leads to a diuretic response similar to that of the normal subject (1, 4).

It would thus appear that the patient with cirrhosis and with a hypotonic milieu who is able to vary urinary concentrations through wide ranges is either releasing ADH in response to a stimulus other than tonicity [such as changes in effective extracellular fluid volume (2, 3)], or that the responses to changes in osmotic pressure are regulated about a lower than normal tonicity. This study is concerned with patients with decompensated cirrhosis who maintain a low serum osmolality, and only those who are able to achieve a diuresis in response to hydration. It was the purpose to determine if, while such patients were undergoing a water diuresis, returning the serum osmolality to its original low level would result in the release of ADH, as reflected by alterations in the flow and concentration of the urine.

METHODS

Subjects selected for study were patients with decompensated cirrhosis associated with the prolonged and excessive intake of alcoholic beverage. All had tense ascites with or without peripheral edema, mild to moderate elevations of serum bilirubin, and a persistently depressed serum osmolality.[1] All were free of cardiovascular and renal disease, and all had previously demonstrated the ability to produce a concentrated as well as a dilute urine. Only subjects able to achieve a flow of urine greater than 6 ml. per minute during the water load were studied. The group consisted of five male and two female patients.

Each study was begun in the morning at least one hour after a light breakfast, and where possible the study was carried out in a private room. Smoking was prohibited after the preceding evening, and the subjects remained recumbent throughout the procedure. All samples of urine were collected through an indwelling urethral catheter which was inserted on the morning of the study. Infusions were delivered through a needle inserted into a forearm vein or through a polyethylene catheter inserted into an antecubital vein. Samples of blood were collected through an indwelling Cournand needle inserted into a forearm vein of the opposite arm. To minimize the influence of emotional reactions on the results of the study the catheterization and venipunctures were performed at least 45 minutes prior to beginning the study. After an initial collection of urine and blood, each subject received a water load as an infusion of 4 per cent hexose (either invert sugar or fructose) solution alone, or in combination with tap water by mouth. The total gain of water was individualized in each subject so that high rates of urinary flow were achieved and

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[1] Determinations of the osmolality of the serum of 16 healthy adults ranged from 281 to 294 mOsm. The range of osmolalities of the sera of subjects chosen for study was 266 to 272 mOsm.

maintained. Fifteen hundred ml. was given during the first hour, and thereafter the infusion was regulated to deliver water in amounts equal to the flow of urine plus an additional 50 ml. per hour for insensible losses. When rates of urinary flow greater than 6 ml. per minute had been reached and maintained for a period of 45 to 60 minutes the infusion was changed to either 3 per cent or 5 per cent sodium chloride. The total amount of sodium chloride given was calculated to correct but not exceed the dilution effect of the water load. This was given rapidly over a period of 15 to 30 minutes. The infusion was then continued as 4 per cent hexose solution at a rate equal to the rates of urinary flow and estimated insensible losses. The positive balance of water was not allowed to fall below that which existed at the time of the maximal water diuresis.

Samples of blood were collected during the period of dilution and maximal urinary flow, midway through the infusion of hypertonic sodium chloride, and during the periods of diminishing urinary flow. The total volume of blood withdrawn prior to the infusion of saline was not in excess of 60 ml. in any subject. Samples of urine were collected at intervals of 10 to 20 minutes, the shorter intervals being necessary to avoid distention of the bladder during high rates of urinary flow. Bladder rinsing was not employed. All samples of serum and urine were frozen immediately.

Total solute concentrations in serum and urine were determined by freezing point depression using a Fiske osmometer. Concentrations of sodium were determined with an internal standard flame photometer. Creatinine concentrations in serum and urine were determined by the method of Hare (8). Calculations of the endogenous clearance of creatinine and the rate of sodium excretion were determined from periods when the flow of urine and the excretion of total solutes were stable in order to minimize the effect of urinary tract dead space and delay time.

TABLE I

Data pertaining to three subjects who experienced discomfort during the infusion of hypertonic NaCl

Subject	Elapsed time	Urine flow	Urine conc.	Serum conc.	Infusion
	min.	*ml./min.*	*mOsm./L.*	*mOsm./L.*	
A	−30–0	0.7	259	269	800 ml. water P.O. and 1,250 ml. 4% invert sugar I.V.
	0–15	5.9	126		
	15–30	7.3	84	272	
	30–45	14.0	57		
	45–60	12.5	55	261	
	60–75	11.5	58	268	230 ml. 5% NaCl
	75–90	7.1	52		
	90–105	0.6	262	274	75 ml. 4% invert sugar
	105–120	0.8	323	272	
B	−30–0	0.7	347	270	760 ml. water P.O. and 1,450 ml. 4% invert sugar I.V.
	0–30	4.3	82		
	30–45	5.3	78	258	
	45–60	5.8	69		
	60–75	5.8	50	255	
	75–90	6.3	44		
	90–105	6.4	40	253	
	105–120	8.7	30	271	370 ml. 5% NaCl
	120–135	3.0	53	274	
	135–150	0.5	320	271	50 ml. 4% invert sugar
	150–165	0.5	453	268	
C*	0–15	2.7	305	266	600 ml. water P.O. and 1,500 ml. 4% fructose I.V.
	15–30	3.6	294		
	30–45	5.4	238		
	45–60	7.5	168	253	
	60–75	6.3	157		380 ml. 5% NaCl
	75–90	2.0	332		150 ml. 4% fructose
	90–105	2.5	360	262	

* Subject C received the water load as an infusion of 4 per cent fructose solution, and the samples of urine during the period of dilution gave a 4-plus reaction with Clinitest. The presence of fructose in these samples probably would have prevented minimal dilution even in the absence of ADH. All other subjects received 4 per cent invert sugar solution, and reducing substance was not detected in other urine samples.

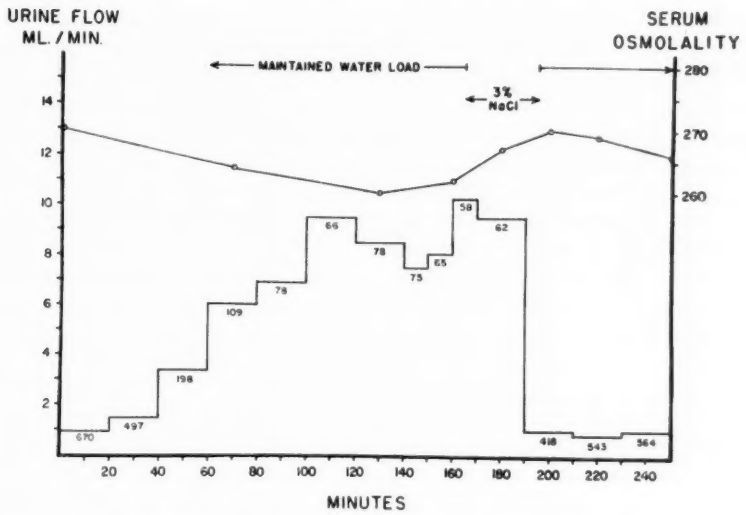

FIG. 1. WATER DIURESIS FOLLOWED BY ANTIDIURESIS WHEN SERUM OSMOLALITY IS RETURNED TO THE INITIAL LOW LEVEL (SUBJECT F)

The line graph at the top represents osmolality of the serum. The bars below represent rates of urine flow. Figures superimposed on the bars denote osmolalities of the respective urine samples. The positive balance of water was maintained constant after the first hour as shown, and the period of infusion of hypertonic saline is indicated.

RESULTS

One subject was eliminated from the results of the study because of a spontaneous antidiuresis which occurred in association with observed emotional stress. Of the remaining six subjects, three experienced mild discomfort at the site of the infusion of hypertonic sodium chloride, and it cannot be concluded with certainty that the observed response was due to a change in tonicity and not to the distress associated with the infusion (9). Data pertaining to these subjects are summarized in Table I. The subjects who recived the infusions through venous catheters did not perceive the change from 4 per cent invert sugar solution to hypertonic sodium chloride, and no stimulus other than the changing tonicity was apparent. The data pertaining to these subjects are given in detail in Table II. Figure 1 graphically represents a typical subject from this latter group. The results are qualitatively the same in each of the six studies.

Lowering the osmolality of the serum 7 to 17 mOsm. as a result of dilution by the water load was associated with a rise in the rate of urinary flow and a fall in the osmolality of the urine in all six subjects. The peak rates of urinary flow ranged from 6.4 to 14.0 ml. per minute, and the minimal total solute concentrations ranged from 30 to 58 mOsm. (except Subject C who had hexosuria during the diluting phase and who had a minimal urinary concentration of 168 mOsm). Restoration of the osmolality of the serum to the pre-experimental levels by the hypertonic infusion was followed by a prompt decrease in the rate of urinary flow and rise in urinary concentration. In each subject small volumes of concentrated urine were produced during the first collection period following the infusion of sodium chloride. Maximal urinary total solute concentrations during the antidiuresis ranged from 323 to 544 mOsm. In each instance the hypertonic NaCl returned the total solute concentration of the serum to within 3 mOsm. of the initial level. During the study of Subject B the osmolality of the serum exceeded the initial level by 4 mOsm. during the infusion of hypertonic saline, but in no subject did the con-

TABLE II

Data pertaining to three subjects who attained water diuresis followed by antidiuresis without discomfort

Subject	Elapsed time	Urine flow	Urine conc.	Serum conc.	Serum sodium	Renal excretion rate of		Clearance of creatinine	Infusion
						Total solute	Sodium		
	min.	*ml./min.*	*mOsm./L.*	*mOsm./L.*	*mEq./L.*	*μOsm./min.*	*μEq./min.*	*ml./min.*	
D	−30–0	0.6	543	272	128	326	16.8		950 ml. water P.O. and 1,450 ml. 4% invert sugar I.V.
	0–15	0.7	599			419		104	
	15–30	0.6	576			346			
	30–45	1.0	368			368			
	45–60	3.8	130			494			
	60–75	11.5	66	262		759			
	75–90	13.2	52			689			
	90–105	11.7	57	261	123	667	30.4	128	
	105–120	8.3	72	260		598			360 ml. 5% NaCl
	120–135	1.5	384	270		576			
	135–150	1.3	544	271	129	707			75 ml. 4% invert sugar
	150–165	0.7	511	273		358	14.7	125	
E	−35–0	0.9	437	270	131	393	24.5	200	2,600 ml. 4% invert sugar I.V.
	0–20	0.8	439			351			
	20–40	1.5	335			503			
	40–60	3.4	135			459			
	60–80	5.4	86			465			
	80–100	7.1	65			462			
	100–125	9.0	49	264		441			
	125–145	7.3	58	265	128	448	34.7	221	
	145–165	3.0	98	263		294			250 ml. 5% NaCl
	165–185	1.8	207	268	128	373			
	185–205	1.1	318			350			200 ml. 4% invert sugar
	205–225	1.3	314			408	79.3	201	
	225–245	1.2		268					
	245–270	1.6	354	271	131	566			
F	−35–0	0.7	692	270	131	488			2,400 ml. 4% invert sugar I.V.
	0–20	0.9	670			570	1.6	161	
	20–40	1.4	497			662			
	40–60	3.3	198			644			
	60–80	6.0	109	264		654			
	80–100	6.9	78			538			
	100–120	9.5	66			624			
	120–140	8.5	78	260		643			
	140–150	7.5	75			563			
	150–160	8.2	65	262	128	533			
	160–170	10.2	58			592	7.1	145	520 ml. 3% NaCl
	170–190	9.6	62	267	132	595			
	190–210	1.0	418	270		418			150 ml. 4% invert sugar
	210–230	0.8	543	269		415			
	230–250	1.0	564			564			
	250–270	1.0	552	266	131	552	13.2	159	

centration reach the normal range of serum osmolality.

The endogenous clearance of creatinine was determined in three subjects. In one subject there was a small rise which persisted after hydration, in one there was a small rise during hydration and a return to the initial level during the antidiuresis, and in the third subject there was no significant change throughout the procedure. These values were all within normal limits.

DISCUSSION

The ability of the decompensated cirrhotic to excrete a standard intake of water has been quantitatively studied and reported elsewhere (1, 4). Many may achieve varying degrees of diuresis,

but occasionally the decompensated cirrhotic may be unable to produce a urine more dilute than plasma (2, 3). The water loads given here were intended primarily to produce, where possible, an obvious water diuresis, and no conclusions can be made regarding the variation in diuretic responses. Only subjects who underwent a water diuresis were studied, and thus conclusions are applicable to *this group only.*

In normal subjects changes in effective osmotic pressure of the body fluids result in changes in the release of ADH. Lowering the osmotic pressure by the gain of water results in an inhibition of the release of ADH and a loss of body water through the excretion of urine of lowered total solute concentration. Elevation of the osmotic pressure above normal by the loss of water or by the gain of effective solute (such as NaCl and other substances which do not readily cross the cell membrane) results in the release of ADH and the conservation of body water by the excretion of a concentrated urine. In this fashion the osmoreceptor-ADH system maintains the tonicity of the body fluids within narrow limits (5–7). Besides pharmacologic and psychic stimuli (10), another apparent mechanism concerned in the release of ADH appears to be related to changes in the extracellular fluid volume or some function thereof (9, 11–14).

The subjects reported here produced large volumes of dilute urine in association with lowering the osmotic pressure of the serum. Restoration of the original osmotic pressure, without a fall in total body volume, resulted in prompt antidiuresis. That these changes in urinary flow and concentration were probably due to changes in the release of ADH seems clear from the character of the urine. There were no marked variations in the rates of solute excretion, and it is unlikely that abrupt changes in the rates of glomerular filtration occurred. In the absence of such changes the observed differences in the flow and concentration of urine may be interpreted as the result of variations in the release of ADH (15, 16). Without pharmacologic and psychic stimuli and with total body volume constant, these changes in the release of ADH seem clearly related to the changes in tonicity and its consequent effects. It may be concluded that at the time these subjects were studied they were sensitive to changes in osmotic pressure, and that the balance of water was regulated to maintain a fixed, although lower than normal, tonicity of the body fluids.

These studies shed no light on the mechanisms responsible in such subjects for a lowered tonicity of the body fluids. In other situations it has been observed that excessive ADH activity may result in the retention of water in excess of solute and thereby lead to hypotonicity (17, 18). It has been suggested that in cirrhosis of the liver decreased hepatic inactivation of the hormone may be a source of excessive antidiuretic activity (19–21), and likewise a decrease in effective extracellular fluid volume could be a stimulus for inappropriate release of the hormone. However, it would not appear that a constant high level of antidiuretic activity exists in subjects who are able to alter abruptly the rates of excretion of water such as those reported here and those of other reports where prompt responses to water loads were observed (1, 4). This subject has been critically reviewed recently by Papper (3).

Such a lowered setting of the osmoreceptor-ADH system as demonstrated by the subjects reported here may possibly be related to a diminished amount of intracellular osmotic activity as has been suggested in other types of chronic hyponatremia (22). The results observed in this study are consistent with such a concept, but confirmation must await accurate volume determinations of the various fluid compartments in these and other states associated with altered osmotic pressure of the serum.

SUMMARY

The antidiuretic effect of hypertonic sodium chloride given during water diuresis was studied in six subjects with decompensated Laennec's cirrhosis and chronically depressed osmolality of the serum. It was found that marked antidiuresis ensued when the osmolality of the serum was restored to the initial low level without a change in the total volume of water in the body. It is concluded that these subjects with low serum sodium and total solute concentration were sensitive to further changes in tonicity, and that the release of antidiuretic hormone was regulated in a qualitatively normal fashion about a lower than normal level of osmolality.

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STUDIES ON THE CIRCULATING INSULIN INHIBITOR FOUND IN SOME DIABETIC PATIENTS EXHIBITING CHRONIC INSULIN RESISTANCE

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Chronic insulin resistance in the diabetic patient has been arbitrarily defined as existing when the daily requirement for insulin exceeds 200 units in the absence of complicating infection or ketoacidosis (1). The general problem of insulin resistance has been the subject of several review articles (1–3). Glen and Eaton first demonstrated that serum obtained from an insulin-resistant diabetic patient protected rabbits from insulin-induced hypoglycemia (4). Subsequently, the studies of Lowell (5) and Lerman (6) suggested the probable antibody nature of this humoral insulin antagonist. Marsh and Haugaard detected an insulin inhibitor in the sera of four diabetic patients requiring at least 300 units of insulin per day (7). In their experiments the ability of insulin to augment glycogen deposition by the isolated rat hemidiaphragm was utilized as the test system. The serum factor was effective only if it, insulin and the hemidiaphragm were in contact simultaneously. More recently there have been several reported cases of chronic insulin resistance in which the insulin antagonist was localized in the γ-globulins and presumed to be an antibody (8–13). Colwell and Weiger (13), in an extensive study of such a patient, noted a correlation between the titer of the insulin antagonist and the serum γ-globulin levels.

Berson and co-workers have demonstrated the presence of an insulin binding protein in the sera of diabetic patients who have received exogenous insulin for at least three months (14). Electrophoretically this protein migrated with the fastest moving γ-globulins and had many characteristics of an insulin antibody. Similar binding of insulin was not observed in the sera of diabetic patients who had never received insulin treatment, but was found in nondiabetic psychiatric patients who had been given insulin shock therapy. Furthermore, the rate of disappearance of insulin-I^{131} injected intravenously was found to be decreased in some diabetic patients (14, 15). Berson and associates observed that patients receiving exogenous insulin were the ones who demonstrated the decreased rate of disappearance (14). Although the binding of insulin to rat hemidiaphragm was diminished in the presence of such sera, no definite correlation existed between the insulin requirement of a patient and the amount of this insulin binding protein (15). However, increased insulin binding by plasma in cases of chronic insulin resistance has been reported previously (16, 17).

Recently adrenocorticotropin (ACTH) and adrenal steroids have been used in the treatment of chronic insulin resistance with variable results (10, 12, 18–20). Howard (18) successfully treated one case whose serum contained an insulin antagonist (7). At a time when the insulin resistance was no longer apparent clinically, antagonist could still be detected in the serum by the rat hemidiaphragm assay, although later it was no longer demonstrable.

Previously we have demonstrated a circulating insulin antagonist associated with diabetic acidosis (21, 22) which is quite distinct from the humoral insulin antagonist demonstrable in some chronically insulin-resistant diabetic patients not in acidosis. The present paper summarizes work which has been done concerning the insulin inhibitor occurring in chronic insulin resistance.

METHODS AND MATERIALS

The method for assaying insulin antagonism in the rat hemidiaphragm was the same as reported previously (21). In these studies the effect of 0.2 unit of insulin on the glycogen deposition by the rat hemidiaphragm in the presence of 1 ml. of serum was measured. The insulin effect using normal serum was 5.35 μM glycogen (glucose equivalents) per Gm. of tissue.

Insulin antagonist was also detected by the ability of

the test serum to protect fasted mice from insulin-induced hypoglycemic death. Albino mice of both sexes weighing 14 to 16 Gm. were used. Sixteen hours prior to an assay, the mice were placed in a constant temperature warm room (37° C.) and given water *ad libitum*, but no food. At the beginning of an assay, water was withdrawn and each mouse was injected intraperitoneally with 1 ml. of serum containing 0.07 unit of insulin and then observed for the following four hours. At least six mice were injected with each serum sample and appropriate controls were included using 1 ml. of normal serum and 1 ml. of normal serum containing 0.07 unit of insulin. The serum samples were previously dialyzed overnight against 0.9 per cent saline to reduce their glucose content.

The γ-globulin levels of the sera were determined by paper electrophoresis using the method of Jencks, Jetton and Durrum (23). The dry paper strip patterns were evaluated by transmission densitometry. Dr. John Fahey of the National Cancer Institute kindly performed these determinations.

Binding of insulin to serum was measured according to the method of Berson and co-workers (14) based on the technique of starch block electrophoresis (24). Two-tenths unit of insulin-I^{131} (Abbott Laboratories) was mixed with 1 ml. of serum and incubated at room temperature for 30 minutes. The insulin-I^{131} was dialyzed for 24 hours against distilled water prior to its use. At the end of the incubation, 1 drop of bromphenol blue solution was added and 0.2 ml. of the mixture was placed in a narrow slit in a starch block. The bromphenol blue served to mark the electrophoretic migration of albumin. The serum on the starch block was then subjected to electrophoresis for 20 hours at 4° C. using 0.05 ionic strength barbital buffer (pH 8.6) and a constant voltage of 350 volts. After the electrophoresis, the starch block was partially dried and then cut up into 1 cm. segments, each of which was suspended in water and was centrifuged. The clear supernatant fluid was decanted, made up to 5 ml., and counted in a well-type scintillation counter. In addition to the bromphenol blue marker for serum albumin, the protein fractions were located by protein determinations according to the modified method of Folin (25). The per cent of the radioactivity which was present in the fastest moving γ-globulin region (representing bound insulin) and just behind the albumin (representing free insulin) were determined. The procedure for the measurement of the effect of serum on the binding of insulin to hemidiaphragm had been described previously (22).

The human insulin containing 1.8 units per mg. was prepared by Dr. Frank Tietze in this laboratory. The sera samples were obtained before the morning insulin injection whenever possible. They were stored at −20° C. until the various analyses could be done. Storage for several months did not seem to have any deleterious effect on the insulin inhibitor or change the binding capacity of the sample. We are indebted to the following for samples of sera: Dr. James Moss, Georgetown University Medical School; Dr. E. O. Friedlander, Veterans Administration Center, Togus, Maine; Drs. James Craig and Max Miller, Western Reserve Medical School; Dr. James Wyngaarden, Duke University School of Medicine; Dr. R. K. Kiskaddon, Youngstown, Ohio; Dr. William Morgan, New York Hospital; and Dr. Reed Harwood, Boston, Mass.

RESULTS

The results of the various assays for insulin antagonist in the sera of these chronically insulin-resistant patients are summarized in Table I. Although 1 ml. of serum from two of the patients (J.C. and M.W.) inhibited the effect of 0.2 unit of insulin on the rat hemidiaphragm, there was no particular correlation between the presence of antagonist and the daily insulin requirement. Since 0.07 unit of insulin was used in the fasted mouse assay, as compared to 0.2 unit in the hemidiaphragm preparation, it was possible to detect circulating insulin inhibitor in the serum of two other insulin-resistant patients (R.C. and C.J.) by the more sensitive mouse assay when no such activity was demonstrable by the hemidiaphragm method. In the serum of some patients receiving up to 2,000 units of insulin per day, no circulating antagonist was found.

Table I also indicates that the sera of all but one of the chronically insulin-resistant patients tested bound augmented amounts of insulin to the fastest moving γ-globulins. In confirmation of the results of Berson and associates (14) binding of insulin to such γ-globulins was observed only in serum from diabetic patients who had received exogenous insulin for at least three months. The per cent of insulin bound to this protein fraction varied from 0.02 to 6 per cent in seven insulin-sensitive diabetics while it exceeded this amount in all of the chronically insulin-resistant patients except one (R.S.). Sera from the four cases with antagonist (J.C., M.W., R.C. and C.J.) bound more than 80 per cent of the added insulin. The amount of insulin used in these experiments, 0.2 unit per ml. of serum, is much greater than the physiologic concentration (26). There did not appear to be any relationship between the daily insulin requirement and the amount of insulin bound. Although the sera were usually obtained before the morning insulin injections, it is possible that in patients receiving such large

TABLE I

Summary of studies assaying serum from diabetic patients with chronic insulin resistance for insulin antagonist

Patient	Date	Daily insulin dose	Diaphragm assay insulin effect μM glycogen (glucose equivalent)/ Gm. of tissue	Mouse assay	Insulin binding		Serum γ-globulin content
			Mean ± S.E.M.	*% Mortality*	*% Bound*	*% Free*	*Gm. %*
J. C.	12–19–56	375	0.58 ± 0.66 (3)*		99	1	2.9
J. C.	12–23–56†	30	5.22 ± 1.83 (3)		97.3	2.7	2.1
M. W.		190	1.45 ± 1.25 (6)		97.5	2.5	2.4
R. C.	9–26–56	1,000	4.98 ± 0.86 (3)	14 (7)	98	2	
R. C.	1–18–57	220	6.39 ± 2.99 (3)		41.8	58.2	1.7
C. J.		615	7.28 ± 2.40 (3)	14 (7)	87	13	1.2
L. S.		230	5.00 ± 0.70 (3)	86 (7)	74	26	
S. S.		285	6.37 ± 0.99 (3)	100 (7)	26.9	73.1	1.4
M. M.		2,000	6.48 ± 0.22 (3)	100 (7)	24	76	2.9
I. H.		200	9.32 ± 1.51 (3)	100 (7)	20.8	79.2	1.5
R. H.		185	6.36 ± 1.77 (3)	86 (7)	9.5	90.5	1.3
R. S.		2,000	8.02 ± 0.28 (3)	67 (6)	1.8	98.2	
Noninsulin-resistant diabetics		10–75			0.02–6.0	94–99.9	
Normal serum			5.35 ± 0.48 (23)	84 (49)	0	100	0.68–1.86

* Number of determinations.
† Following successful ACTH therapy.

doses of insulin a certain amount of insulin might still be present in the sera and interfere with the binding of the insulin added *in vitro*. Some of the insulin-resistant patients had elevated serum γ-globulin concentrations, although there was not always a direct correlation among these levels, the insulin requirement, and the ability of the serum to bind insulin.

Sera obtained from Patient J.C. over a two month period including a successful course of ACTH therapy were studied (Table II). The first sample tested, obtained on Oct. 20, 1956, when her insulin requirement was 180 units per day, did not contain insulin inhibitor by the rat hemidiaphragm assay. By Dec. 15, 1956, her insulin requirement had increased to 375 units per day and now insulin antagonist could be detected. However, when this same serum was assayed by exposing the hemidiaphragm first to serum and then subsequently to insulin, insulin antagonist was no longer demonstrable. This observation is in agreement with the earlier results of Marsh and Haugaard (7). On Dec. 19, 1956, when insulin inhibitor was still present, ACTH administration was begun in a dosage of 60 units per day with a resultant decrease in her insulin requirement to 30 units per day on the fifth day of therapy. At this time (Dec. 23, 1956) her serum no longer inhibited insulin. Serum obtained on Dec. 19 bound 99 per cent of 0.2 unit insulin per ml. and the γ-globulins were elevated to a concentration of 2.9 Gm. per cent (Table I). Although the adrenocorticotropin therapy abolished the insulin resistance and reduced the γ-globulin level to 2.1 Gm. per cent, there was essentially no change in the binding capacity of the serum, a somewhat unexpected finding.

TABLE II

Serial determinations for insulin antagonist in serum from a patient (J. C.) with chronic insulin resistance

Date serum obtained	Insulin requirement	Amount	Insulin effect μM glycogen (glucose equivalent)/Gm. of tissue
	units/day	*ml.*	*Mean ± S.E.M.*
10–20–56	180	1	5.38 ± 1.40 (3)*
12–15–56	375	1	1.70 ± 0.87 (6)
12–15–56 Pre-incubation of serum and diaphragm	375	1	7.64 ± 0.92 (3)
12–19–56	375	1	0.58 ± 0.66 (3)
12–23–56 Following 4 days of ACTH therapy	30	1	5.22 ± 1.83 (3)

* Number of determinations.

The ability of the insulin antagonist in chronic insulin resistance to inhibit human insulin was also demonstrated (Table III). Although there was some reduction in the amount of insulin bound to rat hemidiaphragm in the presence of serum from J.C. as compared to normal, it was not significant (Table IV).

TABLE III

Ability of humoral insulin antagonist from patient with chronic insulin resistance to inhibit the effect of human insulin

Serum	Type of insulin		Insulin effect μM glycogen (glucose equivalent)/Gm. of tissue
		unit	*Mean ± S.E.M.*
J. C. 12–19–56	Beef-pork	0.2	0.58 ± 0.66 (3)*
J. C. 12–19–56	Human	0.2	1.75 ± 0.95 (3)
Normal	Human	0.2	6.48 ± 0.06 (3)

* Number of determinations.

DISCUSSION

In confirmation of previous reports (4, 5, 7, 8, 10–13, 18–20) a circulating insulin antagonist has been detected in the sera of some, but not all, patients with chronic insulin resistance. Using the fasted mouse as a more sensitive assay, it was possible to demonstrate insulin antagonist in cases where it was not detectable in the rat hemidiaphragm preparation. It is, therefore, conceivable that such antagonist could be found in the serum of most, if not all, of these patients if a sensitive enough assay were employed. In the cases where antagonist was demonstrated there was no particular correlation with daily insulin requirement. In chronic insulin resistance, an insulin antagonist, possessing many of the characteristics of an antibody, has been located in the γ-globulin fraction of the serum proteins (8–13). The finding of exalted binding of insulin to the fastest moving γ-globulins in the present cases of insulin resistance is consistent with the concept that the inhibitor is an antibody. In view of the capacity of some of these sera to bind insulin, it is not surprising that these patients were unresponsive to the usual doses of insulin. However, in the case of R.S. (Table I) there was not any definite increase in the insulin binding ability of the serum. This patient, a lipoatrophic diabetic, usually received 2,000 units of insulin per day to control hyperglycemia and glycosuria. The fact that the use of such massive doses of insulin over a prolonged period of time did not produce augmented insulin binding capacity is contrary to the idea that increased insulin binding by serum is secondary to the administration of large amounts of insulin. This patient's serum has also been studied by Arquilla and Stavitsky using an immunologic technique based on hemolysis of sensitized red cells (27). These investigators found that although there were antibodies to insulin, they were not present in very high titer. It therefore appears that the insulin resistance observed in lipoatrophic diabetes is of some other origin (28).

TABLE IV

Effect of serum from diabetic patient with chronic insulin resistance on binding of insulin to rat hemidiaphragm

Source of serum	Number of experiments	μg. insulin-I^{131} bound per Gm. of tissue
		Mean ± S.E.M.
Normal	6	0.018 ± 0.0013
J. C. 12–19–56	6	0.012 ± 0.0036

The successful treatment of insulin resistance with adrenocorticotropin has been reported (12, 18, 19). However, ACTH therapy has not been beneficial in all cases of chronic insulin resistance, even though a circulating insulin inhibitor has been detected (10, 20). It has also been demonstrated that serum obtained after such therapy no longer inhibited insulin (7). Similar observations were made on Patient J.C. whose insulin resistance was successfully treated with ACTH. During the period when insulin antagonism was demonstrable, 1 ml. of her serum bound 99 per cent of 0.2 unit of insulin. Even after therapy with ACTH, when insulin inhibitor was no longer detected, her serum was still able to bind 97.3 per cent of this amount of insulin. This finding was somewhat unexpected since it was believed that the insulin resistance resulted from the increased insulin binding protein. With the disappearance of clinical insulin resistance, the anticipated reduction in insulin binding was not observed. During ACTH therapy, her serum was capable of binding at least 200 units of insulin per L., yet her daily insulin requirement was only 30 units. This discrepancy could be explained in two possible ways. Although the insulin binding capacity of the serum was virtually unchanged, under the influence of ACTH the insulin-insulin binding protein complex might now be physiologically active. Another possible explanation depends on the findings of Berson and Yalow that there are two rates of dissociation of the insulin-insulin binding protein complex (16). If the ACTH causes a more rapid dissociation of insulin from the insulin binding protein, then it is possible that less insulin would be required for con-

trol of the diabetes even though the actual amount of the insulin binding protein remains the same. It is not possible to conclude from the present information which of these two possibilities is correct. It has also been suggested that a sudden decrease in insulin requirement could follow a period of therapy using large amounts of insulin as a result of an accumulation of high concentrations of bound insulin in the serum (29). It is unlikely that this, rather than ACTH, was responsible for the reduction in insulin requirement of Patient J.C. since her insulin requirement had been gradually increasing up to 375 units per day for a period of two months before ACTH therapy. In addition, even after the ACTH therapy was discontinued, she continued to require only 30 units of insulin daily for several weeks. Then her insulin requirement again began to increase so that steroid therapy was reinstituted.

Lowell reported that the circulating insulin antagonist in his patient with chronic insulin resistance did not protect mice from the hypoglycemic effects of human insulin (30). Furthermore, when human insulin was administered to this patient, there was no evidence of insulin resistance. From these studies he concluded that there was no cross-reaction between human insulin and the antibody resulting from beef or pork insulin. Similar conclusions were obtained by Burrows, Peters and Lowell, who found that human insulin did not displace beef-pork insulin from the insulin binding protein (17). However, using an immunologic technique, Arquilla and Stavitsky did find cross-reaction between human insulin and antibodies prepared using beef or pork insulin (31). The insulin inhibitor in the serum of J.C. antagonized the effect of human insulin just as effectively as beef-pork insulin. The reason for this discrepancy is not apparent at this time, but it is possible that in the preparation of the human insulin used in our experiments, it was modified in some way so that it is different from native human insulin and less species specific. It is of some importance to establish definitely whether native human insulin does react with this insulin binding protein in order to assess its role, if any, as an etiologic factor in some cases of diabetes. At present, it seems unlikely that it is of etiologic importance since insulin binding is not apparent until exogenous insulin has been administered for about three months (14).

Although in the present studies protein fractionation was not done to localize the insulin antagonist, it seems likely from other similar cases that the material was an antibody to insulin (4, 5, 7, 8–13). This antibody, present in cases of chronic insulin resistance, resides in the γ-globulins and is different from the α_1-globulin fraction which we have described in the insulin resistance associated with diabetic acidosis (21, 22). The antagonist in diabetic acidosis is still capable of inhibiting insulin in the hemidiaphragm assay even though it is preincubated with the diaphragm and the insulin subsequently added. As shown in Table II this is not true of the insulin antagonist present during chronic insulin resistance. The α_1-globulin antagonist has been demonstrated in sera from patients who have never had previous insulin therapy while the insulin binding protein has never been found in this situation. Furthermore, the insulin resistance associated with diabetic acidosis is transient compared with that in chronic insulin resistance.

SUMMARY

Serum from 2 of 10 cases of chronic insulin resistance was capable of inhibiting the effect of 0.2 unit of insulin in the rat hemidiaphragm assay. Using a more sensitive system, the fasted mouse, it was possible to detect insulin antagonist in 2 other cases where it could not be demonstrated with the hemidiaphragm preparation. In 9 of the 10 cases studied there was an increased binding of insulin to insulin binding protein as compared to insulin-sensitive diabetic patients. In some cases this γ-globulin binding amounted to over 90 per cent of 0.2 unit of insulin per ml. of serum. In general there was good correlation between the amount of insulin binding and the demonstration of circulating insulin antagonist. Although the total serum γ-globulin level was elevated in some cases, there was no particular relationship among these values, the daily insulin requirement, or the amount of insulin bound. In one patient, treatment with adrenocorticotropin (ACTH) abolished the insulin resistance, and insulin antagonist, previously present, was no longer demonstrable. Contrary to expectations this therapy did not diminish the ability of the serum to bind large

amounts of insulin. The insulin antagonist also inhibited human insulin.

In contrast to the insulin antagonist associated with diabetic acidosis, the inhibitor in cases of chronic insulin resistance is not effective unless the hemidiaphragm, insulin, and serum are all incubated simultaneously.

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CARDIAC, HEMODYNAMIC AND RENAL FUNCTIONS IN CONGESTIVE HEART FAILURE DURING INDUCED PERIPHERAL VASODILATATION; RELATIONSHIP TO STARLING'S LAW OF THE HEART IN MAN * †

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The present study of the hemodynamic effects of induced peripheral vasodilatation had two purposes: one, to determine what changes are produced in cardiac, hemodynamic and renal functions of man in congestive heart failure when vascular pressures are lowered by noncardiac means, and two, to determine whether Starling's law of the heart applies in intact man, especially in congestive heart failure.

Expanding on the observations of Frank (1) on the frog heart, Starling, with Patterson and Piper (2, 3), determined in the isolated heart-lung preparation of the dog that cardiac output increased progressively as ventricular filling pressure (2) and ventricular diastolic volume (3) increased (ascending limb of curve); however, beyond a point considered to indicate a failing myocardium, cardiac output decreased with further increments in ventricular filling pressure or diastolic volume (descending limb of curve). The final generalization derived from these observed relationships Starling (4) then expressed as the law of the heart: "The energy of contraction, however measured, is a function of the length of the muscle fiber." Starling's law has been extrapolated to the intact, humorally and neurogenically homeostatic animal, including man, and has come to dominate present concepts of both normal and abnormal cardiac function. Such extrapolation requires validation.

The applicability of Starling's law to the more intact animal has been investigated by Sarnoff and his collaborators, Berglund, Isaacs, Case and Sarnoff (5–8), in the anaesthetized, open-chested dog with the heart *in situ* and its nerve supply intact but its circulation completely isolated and subjected to measurement. In large measure, the relationships between cardiac work and ventricular filling pressure were similar to the results obtained by Starling. Two modifications evolved: one, ventricular function did not follow a single stroke work-filling pressure curve but rather a series or family of similar curves, each curve determined by the circulatory state at the time (anemia, aortic constriction, coronary artery constriction, induced epinephrinemia); two, a descending limb on the ventricular work-filling pressure curve did not occur in the "normal" satisfactory preparation but appeared when the myocardium was "compromised," as by severe anemia, severe coronary artery constriction or shock. Several features of these experiments, especially the large changes in blood volume required to alter the filling pressures and the relatively short periods during which the altered pressure-flow relationships were determined, still raise the question whether the observed results are applicable to man with normal, and especially failing, circulations in a steady state for long periods.

Several studies suggest that Starling's law may apply in man. McMichael, with Sharpey-Schafer and Howarth (9, 10), and Judson, Hollander, Hatcher, Halperin and Friedman (11), observed an increase in cardiac output (hence cardiac work since arterial pressure did not change) when the elevated intracardiac pressures of subjects in congestive heart failure were lowered by phlebotomy or by inducing venous stasis in the limbs. These observations have the disadvantage that phle-

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botomy reduces blood volume and the cuffs inducing venous stasis can be maintained for periods which may be too short to permit attainment of circulatory equilibrium.

The advent of vasodilator drugs suggested the possibility of lowering ventricular filling pressure by noncardiac means for sufficiently long periods to attain circulatory equilibrium at a lowered pressure level and, thereby, to permit testing of the applicability of Starling's law to man. The present experiments were designed to lower ventricular filling pressure by producing primary peripheral vasodilatation, then observing whether cardiac function altered as predicted by the law of the heart: an increase in cardiac work when the elevated ventricular filling pressure in congestive heart failure was lowered, and a decrease in cardiac work when normal filling pressure was reduced.

PROCEDURES, METHODS AND SUBJECTS

General. The plan of study was to measure all functions simultaneously during a control state, during induced and maintained vasodilatation and again on return of the control state. The drug Arfonad®, a thiophanium derivative [d-3,4(1',3'-dibenzyl-2'-ketoimidazolido)-1,2-trimethylene thiophanium d-camphor sulfonate], was chosen to produce vasodilatation and lower vascular pressures because this drug has no known direct myocardial or cardiac action. Its effects are produced by blocking sympathetic and parasympathetic ganglia and perhaps also by direct peripheral vascular dilatation (12, 13).

Cardiac output was determined by the direct Fick (14) method. Intracardiac and systemic vascular pressures were obtained directly and recorded with strain gages and electronic recorder. Renal hemodynamic functions were measured by clearance techniques (15) and electrolyte concentrations by flame photometer. Standard methods were used to determine the following: oxygen and carbon dioxide in blood (16), room air (17) and exhaled air (17); the concentrations of inulin (18), para-aminohippurate (19) and sodium and potassium (flame photometer) in blood and urine; the plasma and blood volumes (20). Determinations were made in successive periods of 15 to 20 minutes duration, with cardiovascular dynamic measurements made at the midpoint of the periods and renal hemodynamic functions and water and electrolyte excretions measured over the entire period. The nature, timing and sequence of the necessary procedures have been described previously in detail (21, 22). Renal hemodynamics and urinary excretions were determined in only one-half of the experiments.

Procedures. The observations were made in the morning on supine subjects without breakfast. A control electrocardiogram was taken and the urinary bladder was catheterized. The intravenous infusion of inulin and para-aminohippurate was delivered continuously at 1 ml. per minute by a Bowman pump. A double lumen intracardiac catheter was placed with the distal opening in the pulmonary artery and the proximal opening in the right ventricle. A systemic artery was cannulated. A slow intravenous infusion of 5 per cent dextrose in water was begun and the system was provided with a three-way stopcock to permit switching from dextrose infusion to Arfonad® infusion without the subject's knowledge.

After three periods of control measurements the intravenous infusion of Arfonad®, 1 mg. per ml. in 5 per cent dextrose in water, was begun and delivered continuously by an infusion pump. The Arfonad® infusion was started at 0.5 mg. per minute and adjusted at 5 to 10 minute intervals until the desired degree of vasodilatation, as determined by the level of arterial pressure, was obtained. This level was maintained for one hour, during which three periods of determinations were made, with their midpoints 15, 30 and 60 minutes after attaining the desired level of vasodilatation. The infusion of Arfonad® was then discontinued and after the vascular pressures had returned to control levels, or as close as it appeared they would return, the final period of determinations was made. While awaiting return of the control state, blood volume was determined by the Evans blue dye (T-1824) method (20).

When possible, pulmonary "capillary" or "wedge" pressure was obtained during the third control, the third vasodilatation and the one postvasodilatation periods. Right atrial pressure was recorded during vasodilatation only on those occasions when the catheter could not be placed in the pulmonary artery. Right atrial pressure was routinely recorded during removal of the catheter at the close of the observations. Arterial hematocrits were obtained during the control period and again during the postvasodilatation period. Blood oxygen capacity was determined twice, once on samples pooled from the control periods and the second time on samples pooled from the later vasodilatation periods. The corresponding values were used to calculate arterial oxygen saturation for the individual periods.

Even though Arfonad® was infused in small doses, ventricular filling pressure could not be lowered without a concomitant reduction in systemic arterial pressure. Therefore, the dose of the drug was carefully regulated and the fall in systemic arterial pressure was always controlled. In hypertensive patients arterial pressure was lowered to normal, or slightly higher, levels; in normotensive subjects systolic arterial pressure was lowered to 100 to 110 mm. Hg. These pressures served as criteria for the desired degree of vasodilatation and were maintained for one hour. Circulatory collapse and severe hypotension were specifically avoided.

Calculations. Standard calculations were used, at times modified as required by substitutions and assumptions which led to the actual formulations used. Surface

area was always based on edema-free weight. When measured right atrial pressure was not available, its value was taken to be the corresponding right ventricular end diastolic pressure minus the difference observed between right ventricular end diastolic pressure and right atrial pressure measured when the catheter was removed at the end of the observations. In occasional instances an available right atrial pressure was substituted for an unavailable right ventricular diastolic pressure. In calculations requiring pulmonary "wedge" pressure (pulmonary vascular resistance and left ventricular minute and stroke work) the following substitutions were made in those periods where this measurement was not available: The single value obtained during vasodilatation was used in calculations for both the maximum vasodilatation period and the average of the vasodilatation periods; values obtained during the control period or the postvasodilatation period were substituted for each other when one of the measurements was not available.

The formulae used were: systemic vascular resistance $=(SA_m - RA_m) \div C.O.$; pulmonary vascular resistance $=(PA_m - PC_m) \div C.O.$; renal vascular resistance $=(SA_m - RA_m) \div RBF$. Vascular resistances are expressed as mm. Hg per L. per minute, since nothing is gained by the more elaborate calculation into fundamental units.

Left ventricular minute work index $=(SA_m - PC_m) \times C.I. \times 14.4 \div 1{,}000$; right ventricular minute work index $=(PA_m - RV_d) \times C.I. \times 14.4 \div 1{,}000$; stroke work index = minute work index of corresponding ventricle $\times 1{,}000 \div V.R.$ Minute work index is expressed as Kg. M. per $M.^2$ per minute and stroke work index as Gm. M. per $M.^2$ per beat.

The symbols are defined as follows: SA_m = mean systemic arterial pressure, mm. Hg; RA_m = mean right atrial pressure, mm. Hg; PA_m = mean pulmonary arterial pressure, mm. Hg; PC_m = mean pulmonary "wedge" pressure, mm. Hg; RV_d = right ventricular end diastolic pressure, mm. Hg.

C.O. = cardiac output, L. per minute; C.I. = cardiac index, L. per $M.^2$ per minute; RBF = renal blood flow in L. per minute = renal plasma flow $\div$ (1-Hct), where Hct = hematocrit, expressed as a decimal; V. R. = ventricular rate, per minute.

RESULTS

Subjects. Table I presents clinical information regarding the 17 patients on whom 21 experiments were performed. The subjects have been divided into four groups: Group I, congestive heart failure—five normotensive and five hypertensive cardiac patients with typical clincial congestive heart failure indicated by low cardiac indices and high A-V oxygen differences; Group II, partial cardiac compensation—two normotensive and two hypertensive cardiac patients clinically recovered from congestive heart failure by appropriate therapy and without signs or symptoms of heart failure but with below normal cardiac indices and A-V oxygen differences above 6 volumes per cent; Group III, noncardiac and cardiac compensation—two noncardiac subjects convalescing from pneumonia and alcoholism, respectively, and three cardiac patients, one normotensive and two hypertensive, fully compensated from congestive heart failure, receiving maintenance doses of digitalis, without signs and symptoms of heart failure and with normal cardiac indices and A-V oxygen differences; Group IV, "normal output" congestive failure— two subjects, one normotensive and one hypertensive, with signs and symptoms of congestive heart failure but with normal cardiac indices and normal arterial-mixed venous oxygen differences. The four subjects, B.K., L.R., S.T. and E. S., who were studied twice, once during congestive heart failure and again after recovery of compensation, appear in two groups according to their cardiovascular status on each occasion.

General response. There were no apparent differences between the subjects with congestive heart failure and the compensated cardiac and control subjects with respect to the amount of Arfonad® required to maintain vasodilatation, the speed with which vasodilatation occurred and the rapidity of return to the control state after stopping the drug (Table I). The dosage range was 0.35 mg. to 1.25 mg. per minute with the usual dose between 0.5 mg. to 1.0 mg. per minute. Two subjects (J.H. and J.M.), both of Group III, required much larger amounts, 3.0 and 2.0 mg. per minute, respectively. The rate of infusion of the drug was often increased by 0.25 mg. to 0.5 mg. per minute during the last half hour of vasodilatation in order to prevent the blood pressure from rising above the desired level. The time required to reach the desired level of vasodilatation varied considerably but averaged 25 to 30 minutes. The control state was reached on an average of 30 minutes after stopping the infusion.

During the vasodilatation the subjects with congestive heart failure seemed somewhat improved. They were quieter, less restless and less complaining. Cerebration seemed less active and they even dozed. Respirations were often more shallow and irregular. Yawning occurred infrequently.

TABLE I

Grouping and clinical status of patients and dosage of Arfonad®

Patient	Sex	Age	Surface area	Diagnosis*	Congestive heart failure							Therapy before test	Plasma volume	Hematocrit	Blood volume	Arfonad® infusion		
					Times in failure	Degree	Edema	Liver	Veins	Rales	Dyspnea					Amount	Time reach dilatation	Time return control
		yrs.	*M.²*										*L.*	%	*L.*	*mg./min.*	*min.*	*min.*
Group I. Congestive heart failure																		
J. S.	M	62	1.84	ASHD	1	4+	4+	3+	3+	3+	3+	Aminophyllin, 1×	4.85	45	8.82	0.6	50	32
R. R.	M	69	1.83	ASHD	4	4+	4+	4+	4+	3+	4+	Morphine				0.7	126	
G. L.	M	56	1.70	ASHD	3	3+	3+	±	3+	2+	2+	Digitoxin, daily Thiomerin®, 1×	4.21	37	6.68	0.5	55	55
R. A.	M	55	1.34	ASHD	2	3+	3+	3+	3+	1+	3+	Mercuhydrin®, 1× Thiomerin®, 1×	3.66	40	6.10	0.35	20	27
B. K.	M	58	1.79	RHD	4	2+	±	4+	0	0	±	Digitalis, daily Thiomerin®		52		1.0	35	
L. R.	M	56	1.77	HCVD	1	2+	1+	0	0	0	1	None				0.8	30	25
R. H.	M	45	1.86	HCVD	2	1+	±	±	0	0	±	Digoxin®, 1.5 mg. Thiomerin®, 3×				0.8	30	63
B. K.	M	58	1.79	RHD	4	4+	4+	4+	3+	2+	4+	Digitalis, daily Mercuhydrin®		44		0.7	32	25
W. C.	M	41	1.65	HCVD	2	2+	0	3+	2+	0	1+	Digoxin®, daily	2.54	46	4.68	1.0	47	43
S. T.	M	45	1.94	HCVD	2	3+	3+	4+	3+	2+	3+	Digoxin®, 1.5 mg. Mercuhydrin®, 2×	5.52	27	7.55	1.25	47	58
Group II. Partial compensation																		
J. J.	M	51	1.54	UnkHD	3	1+	0	0	0	0	1+	Bed rest		45		0.85	25	
W. G.	M	76	1.51	ASHD	1	0	0	0	0	0	0	Digitalis, daily				0.4	41	30
L. R.	M	56	1.77	HCVD	1	0	0	0	0	0	0	Digitalis, daily				1.1	41	50
S. T.	M	45	1.94	HCVD	2	±	±	2+	0	0	±	Digitalis, daily	4.57	30	6.53	1.0	45	46
Group III. Noncardiac and compensated cardiac																		
L. K.	M	48	1.75	Post alcoholism	0	0	0	3+	0	0	0	Hexavitamins		35		0.35	37	40
J. H.	M	62	1.75	Post pneumonia	0	0	0	0	0	0	0	None	3.53	37	5.62	3.0	54	26
J. M.	M	52	1.75	UnkHD	1	0	0	1+	0	0	0	Digitalis, daily	4.60	35	7.06	2.0	47	29
A. H.	F	77	1.44	HCVD ASHD	1	0	0	0	0	±	0	Digitalis, daily	2.16	40	3.60	0.7	46	33
E. S.	M	66	1.65	HCVD	1	0	0	0	0	0	0	Digitalis, daily	3.75	38	6.05	0.9	48	27
Group IV. "Normal output" congestive failure																		
B. O.	F	76	1.24	ASHD	1	4+	4+	±	3+	2+	2+	Amytal		35		0.4	22	17
E. S.	M	66	1.65	HCVD	1	2+	±	3	0	2	1	Aminophyllin, 1×	3.93	35	6.05	0.6	28	19

* Abbreviations are as follows: ASHD, arteriosclerotic heart disease; RHD, rheumatic heart disease; HCVD, hypertensive heart disease; UnkHD, heart disease of unknown etiology.

When ventricular premature contractions were previously present they became less frequent. Similar reactions occurred in subjects without congestive heart failure and, in addition, restlessness, tension and "inside nervousness" were encountered. After stopping the infusion of the drug, the initial condition of the patient rapidly returned.

Presentation of data. The primary and derived data are too voluminous to be presented for all subjects. The data presented are tabulated for each group of subjects and give the following information for each subject in a group: *a*) the average data of the control periods, *b*) the data of the period of maximum change during vasodilatation, *c*) the average data of the vasodilatation periods, *d*) the data of the postvasodilatation period, then the *average data* for all subjects of the group in the four categories above. The average data of the control (*a*) and vasodilatation (*c*) periods are the averages of three periods except occasionally when experimental difficulties reduced the number to two and once to one. The period of maximum change during vasodilatation was usually the first vasodilatation period and only once later than the second. The difference between the average data of the three vasodilatation periods (*c*) and the data of the period of maximum change (*b*) indicates the failure of the initial changes to be maintained throughout the period of vasodilatation. The shortcomings of the comparison of individual data with average data are recognized. However, the presentation permits comparison of average data from control periods with average data during vasodilatation and comparison of the single "maximum change" period with the single postdilatation period, permitting, thereby, several ways of examining the effects produced by vasodilatation. These abridged data present with validity the changes indicated by the complete data.

Vascular pressures, vascular resistances, ventricular rate (Tables II to VII, Figures 1 and 2)

In all subjects of all four groups the blood pressure during Arfonad® infusion fell in the systemic circuit, the pulmonic circuit and right heart; remained at the lowered level, or rose slightly, during the hour of vasodilatation; and returned to, or well toward, the control levels after stopping the infusion. Although the actual decreases in the various systemic and pulmonic pressures were greater when the control pressures were elevated, the percentile decreases were of the same order of magnitude, usually between 20 and 30 per cent (Table VII), in both the greater and lesser circulations. An exception was the 60 per cent decrease in pulmonary "capillary" pressure in Groups II, III and IV. Although the elevated pressures due to congestive heart failure (right atrial, right ventricular, pulmonary arterial and pulmonary "capillary") were lowered decidedly, they did not fall to the normal range. Qualitatively and quantitatively the same degree of change in vascular pressures occurred in all four groups of subjects, and within each group normotensive and hypertensive subjects responded similarly.

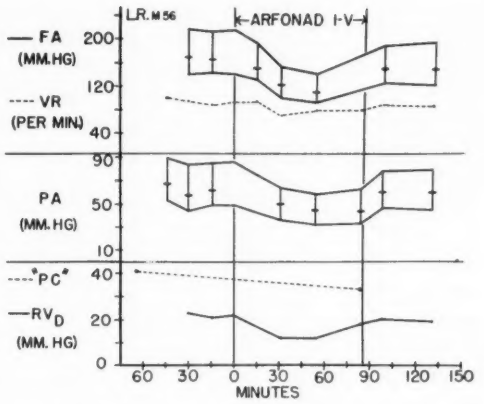

FIG. 1. TYPICAL SERIAL DETERMINATIONS OF VASCULAR PRESSURES IN A REPRESENTATIVE SUBJECT (L.R.) WITH CONGESTIVE HEART FAILURE

Rate of intravenous infusion of Arfonad®, 0.8 mg. per minute. FA designates femoral artery pressure; VR, ventricular rate; PA, pulmonary artery pressure; "PC," pulmonary "wedge" or "capillary" pressure; RV_d, right ventricular end diastolic pressure.

Systemic vascular resistance decreased in all groups of subjects by about 30 per cent during vasodilatation and returned to control levels after the drug was discontinued. Exceptions were a 10 per cent decrease in systemic vascular resistance in the noncardiac and compensated subjects (Group III). Pulmonic vascular resistance, on the other hand, decreased, again by about 30 per cent, only in the the congestive heart

TABLE II

Hemodynamic effects of Arfonad®: Congestive heart failure—Normotensive subjects

Patient*		Cardiac index	A-V O_2 diff.	Arterial oxygen content	Arterial oxygen saturation	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure†	Systemic vascular resistance	Pulmonary "wedge" pressure	Right atrial pressure	Right ventricular pressure	Pulmonary artery pressure†	Pulmonary vascular resistance	Left ventricle		Right ventricle	
																	Minute work index	Stroke work index	Minute work index	Stroke work index
		L./M.²/min.	*Vol. %*	*Vol. %*	*%*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
J. S.	Avg. Cont.	1.64	8.63	15.13	88.6	6.50	142	11.58	68	136/90(105)	28.5		21	63/26						
(1)	Max. Vasod.	2.03	6.92	14.04	81.8	7.12	140	10.80	68	104/68(80)	18.0		13	54/18						
	Avg. Vasod.	1.76	7.51	14.12	82.3	6.61	131	10.27	67	101/67(76)	19.1		14	53/21						
	Post Vasod.	1.41	9.12	15.12	88.2	6.00	128	10.75	63	130/81(101)	31.3		20	60/23						
R. R.	Avg. Cont.	1.40	9.53	16.46	95.6	6.93	134	7.95	136	127/87(105)	39.2	23		41/18	41/26(34)	4.3	1.65	12.2	0.32	2.4
(3)	Max. Vasod.	1.34	8.38	15.36	89.1	6.98	112	7.65	123	93/64(78)	28.5			30/10	28/10(23)	4.1	1.25	10.2	0.25	2.0
	Avg. Vasod.	1.40	8.74	15.48	89.8	6.75	119	8.03	126	107/74(89)	29.1	13		35/15	38/22(29)	6.2	1.53	12.2	0.28	2.2
	Post Vasod.																			
G. L.	Avg. Cont.	1.40	9.96	17.29	92.9	7.42	136	8.49	106	136/90(108)	39.0	16		48/24	49/34(38)	9.2	1.85	17.5	0.28	2.7
(1)	Max. Vasod.	1.75	7.07	15.53	83.4	8.46	123	7.48	93	103/64(77)	20.7			36/17	34/23(25)	3.0	1.54	16.5	0.20	2.2
	Avg. Vasod.	1.41	8.99	16.07	86.3	7.09	122	8.55	100	111/72(85)	30.2	16		39/19	34/25(28)	5.0	1.40	14.0	0.18	1.8
	Post Vasod.	1.24	10.96	17.64	94.8	6.68	135	9.64	108	148/96(110)	44.1			52/22	52/38(43)	12.8	1.68	15.5	0.37	3.5
R. A.	Avg. Cont.	1.73	8.02	17.25	91.4	9.23	138	5.83	86	137/53(83)	29.7			64/17	64/31(43)				0.65	7.5
(2)	Max. Vasod.	2.00	6.56	16.52	87.9	9.94	132	5.85	76	112/42(69)	21.7			56/12	62/28(41)				0.84	11.0
	Avg. Vasod.	1.97	7.00	16.58	88.0	9.57	138	6.19	78	118/44(69)	21.7			58/14	62/30(41)				0.76	9.8
	Post Vasod.	1.71	8.40	17.30	91.7	8.90	144	6.92	81	138/55(83)	30.6		13	59/15	65/35(41)				0.64	7.9
B. K.	Avg. Cont.	1.60	8.26	19.62	91.2	11.36	132	5.89	72	139/87(108)	34.2	25		65/11	72/35(48)	8.1	1.91	26.5	0.85	11.8
(1)	Max. Vasod.	2.21	5.98	18.50	86.0	12.52	131	5.96	68	103/67(81)	19.2			51/6	53/27(38)	6.3	2.16	31.8	1.02	15.0
	Avg. Vasod.	1.89	6.47	18.58	86.4	12.12	120	5.13	65	111/69(86)	24.4	13		50/6	53/26(37)	7.1	1.99	30.6	0.85	13.0
	Post Vasod.																			
Avg.	Avg. Cont.	1.55	8.88	17.15	91.9	8.29	136	7.95	94	135/81(102)	34.1	21[3]		56/19	56/32(41)	7.2[3]	1.81[3]	18.7[3]	0.53[4]	6.1[4]
5	Max. Vasod.	1.87	6.98	15.99	85.6	9.00	128	7.55	86	103/61(77)	21.6			45/13	44/22(32)	4.5[3]	1.65[3]	19.5[3]	0.58[4]	7.6[4]
Pts.‡	Avg. Vasod.	1.69	7.74	16.17	86.6	8.43	126	7.63	87	110/65(81)	24.9	14[3]		47/15	47/26(34)	6.1[3]	1.64[3]	18.9[3]	0.52[4]	6.7[4]
	Post Vasod.	1.45[3]	9.49[3]	16.69[3]	91.6[3]	7.19[3]	136[3]	9.10[3]	84[3]	139/77(98)[3]	35.3[3]			57/20[3]	58/36(42)[3]	12.8[1]	1.68[1]	15.5[1]	0.51[3]	5.7[3]

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Numbers under initials of patients indicate period of maximum effect from Arfonad®.

† Pressures in parentheses are mean pressures.

‡ Averages are for five patients except as otherwise indicated by superscript numbers in parentheses.

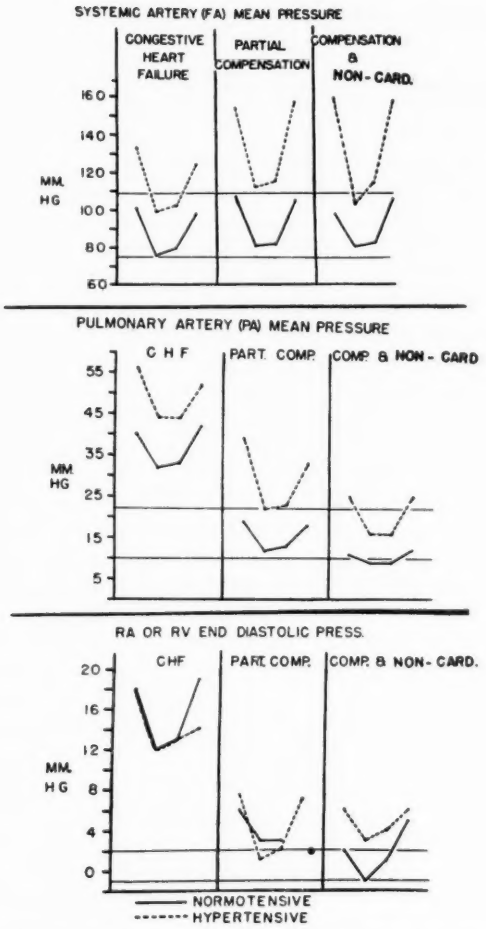

FIG. 2. EFFECT OF INTRAVENOUS INFUSION OF ARFONAD® ON VASCULAR PRESSURES IN CONGESTIVE HEART FAILURE AND IN CARDIAC COMPENSATION

The data are the average data for all subjects in each group. For any line the first point is the average of the control periods, the second point is the period of maximum effect during vasodilatation, the third point is the average of the vasodilatation periods, the fourth point is the postvasodilatation period. In each panel the two horizontal lines indicate the range of normal values.

failure group and increased in each of the other three groups.

Ventricular rate decreased during the vasodilatation by 5 to 10 per cent in all subjects except in Group III where the rate tended to increase slightly, by 5 per cent. Unlike the vascular pressures and resistances, ventricular rate tended to remain below the control level after stopping the Arfonad® infusion.

The data indicate a generally similar decrease in vascular pressures and resistances in all groups of subjects, except for the changes in opposite direction in pulmonary vascular resistance.

Respiration and metabolism (Tables II to VII, Figure 3)

During vasodilatation the rhythm of respiration frequently became irregular but in all four groups of subjects the respiratory rate remained essentially unaltered. The minute volume of respiration showed inconsistent changes, tending to decrease slightly, 6 per cent, in subjects with heart failure (Group I) or partial compensation (Group II) and to increase by a comparable amount in noncardiac and compensated subjects (Group III). Except for lack of change in the two subjects with "normal output" failure (Group IV), the metabolic rate decreased slightly, 6 to 12 per cent, but consistently and significantly ($p = < 0.01$) in the other subjects during vasodilatation and returned to the initial level upon discontinuing the vasodilator drug.

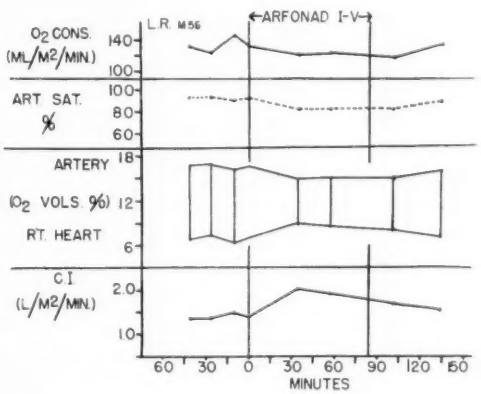

FIG. 3. TYPICAL SERIAL DETERMINATIONS OF BLOOD OXYGEN CONTENT AND CARDIAC OUTPUT IN A REPRESENTATIVE SUBJECT (L.R.) WITH CONGESTIVE HEART FAILURE

Rate of intravenous infusion of Arfonad®, 0.8 mg. per minute. O_2 Cons. designates oxygen consumption; Art. Sat., arterial oxygen saturation; C.I., cardiac index. In the third panel from the top are indicated the oxygen content of blood of artery, top line; of mixed venous blood, lower line; and A-V oxygen difference, vertical connecting lines.

TABLE III

Hemodynamic effects of Arfonad®: Congestive heart failure—Hypertensive subjects

Patient*		Cardiac index	A-V O_2 diff.	Arterial oxygen content	Arterial oxygen saturation	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure†	Systemic vascular resistance	Pulmonary "wedge" pressure	Right atrial pressure	Right ventricular pressure	Pulmonary artery pressure†	Pulmonary vascular resistance	Left ventricle		Right ventricle	
																	Minute work index	Stroke work index	Minute work index	Stroke work index
		L./M.²/min.	*Vol. %*	*Vol. %*	*%*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
L. R.	Avg. Cont.	1.38	9.64	16.66	91.9	7.02	133	10.23	94	213/142(166)	62.9	41		85/22	87/47(62)	8.6	2.48	26.4	0.80	8.5
(1)	Max. Vasod.	2.02	6.00	14.99	82.5	8.99	121	9.63	71	152/100(122)	30.3				64/36(50)	4.8	2.59	36.4	0.99	13.9
	Avg. Vasod.	1.88	6.48	15.10	83.2	8.62	121	9.87	78	160/106(127)	35.0	33	10	66/16	67/38(52)	5.7	2.54	32.6	0.98	12.5
	Post Vasod.	1.55	8.80	16.17	89.2	7.37	137	10.66	83	192/121(151)	51.0			79/19	79/45(60)	6.9	2.46	29.6	0.92	11.0
R. H.	Avg. Cont.	1.80	9.12	22.24	97.8	13.16	166	8.67	115	153/99(119)	33.7		6	27/6						
(1)	Max. Vasod.	1.96	6.74	21.26	94.6	14.52	133	9.76	94	117/77(90)	24.0		2	20/2						
	Avg. Vasod.	1.82	7.53	21.03	93.6	13.50	138	9.13	88	118/77(91)	25.8		2	20/2						
	Post Vasod.	1.72	8.48	21.28	94.7	12.80	148	7.70	88	128/90(101)	30.6		3	27/5						
B. K.	Avg. Cont.	1.36	11.47	18.28	89.6	6.81	153	9.42	102	153/115(133)	47.7	33		83/26	83/43(59)	10.7	1.96	19.4	0.64	6.3
(1)	Max. Vasod.	1.08	10.16	17.35	85.0	7.19	110	6.55	87	126/86(101)	40.6			72/25	76/35(47)	9.3	1.12	12.9	0.34	3.9
	Avg. Vasod.	1.20	10.81	17.60	86.3	6.99	128	7.34	89	130/90(105)	38.6	29		72/24	76/40(52)	10.7	1.31	14.7	0.48	5.5
	Post Vasod.	1.43	9.63	17.11	84.9	7.48	138	7.91	81	133/90(105)	32.4			67/22	67/31(44)	4.3?	1.48	18.3	0.45	5.6
W.C.	Avg. Cont.	1.41	9.38	19.28	93.0	9.90	132	6.55	95	147/100(113)	43.9			78/14	84/41(58)	13.3	1.74	18.4	0.89	9.4
(2)	Max. Vasod.	1.64	7.49	18.17	87.8	10.68	122	6.11	94	118/78(90)	29.9			/12	65/34(46)	9.2	1.63	17.3	0.80	8.5
	Avg. Vasod.	1.52	8.03	18.31	88.3	10.27	122	6.22	94	115/76(88)	31.5	21		/12	62/34(46)	10.0	1.47	15.6	0.74	7.9
	Post Vasod.	1.20	11.05	18.67	90.7	7.62	133	7.00	104	144/98(116)	52.2	27	12	90/17	86/47(63)	18.2	1.54	14.8	0.80	7.6
S. T.	Avg. Cont.	2.59	6.01	11.93	85.0	5.91	156	9.75	107	198/114(137)	23.9	31		59/20	61/38(46)	3.0	3.95	36.9	0.97	9.1
(1)	Max. Vasod.	2.99	4.57	10.80	76.8	6.23	137	9.16	94	161/87(110)	17.2			42/11	44/25(33)	2.1	3.83	40.8	0.95	10.1
	Avg. Vasod.	2.85	4.58	10.46	74.3	5.89	130	9.83	91	154/84(104)	17.0	21		41/13	43/22(30)	1.6	3.40	37.4	0.70	7.7
	Post Vasod.	2.20	6.83	11.68	83.2	4.85	146	10.91	97	191/110(133)	27.3		17	56/21	56/25(43)	2.8	3.23	33.3	0.70	7.2
Avg.	Avg. Cont.	1.71	9.12	17.68	91.5	8.56	148	8.92	103	172/113(133)	42.3	35[3]		66/18	79/42(56)[4]	8.9[4]	2.53[4]	25.3[4]	0.83[4]	8.3[4]
5	Max. Vasod.	1.94	6.99	16.51	85.3	9.52	125	8.24	88	135/86(103)	28.4			45/12[3]	62/32(44)[4]	6.4[4]	2.29[4]	26.8[4]	0.77[4]	9.1[4]
Pts.‡	Avg. Vasod.	1.85	7.49	16.50	85.1	9.05	128	8.48	88	135/87(103)	29.6	26[4]		50/13[4]	62/34(45)[4]	7.0[4]	2.18[4]	25.1[4]	0.73[4]	8.4[4]
	Post Vasod.	1.62	8.96	16.98	88.5	8.02	140	8.84	91	158/102(121)	38.8	27[3]		64/17	72/37(52)[4]	8.0[4]	2.18[4]	24.0[4]	0.72[4]	7.9[4]
Combined averages: Five normotensive and five hypertensive patients with congestive heart failure																				
Avg.	Avg. Cont.	1.63	9.00	17.42	91.7	8.42	142	8.44	98	154/97(118)	38.2	29[5]		61/18	68/37(48)[8]	8.2[7]	2.22[7]	22.5[7]	0.68[8]	7.2[8]
10	Max. Vasod.	1.90	6.98	16.25	85.4	9.26	126	7.90	87	119/74(90)	25.0			45/12[8]	53/27(38)[8]	5.5[7]	2.02[7]	23.7[7]	0.67[8]	8.3[8]
Pts.§	Avg. Vasod.	1.77	7.62	16.34	85.8	8.74	127	8.06	88	122/76(92)	27.2	22[5]		48/14[8]	54/30(40)[8]	6.7[7]	1.95[7]	22.4[7]	0.62[8]	7.6[8]
	Post Vasod.	1.56[5]	9.16[8]	16.87[8]	89.7[8]	7.71[8]	139[8]	8.94[8]	88[8]	151/93(112)[8]	37.5[8]			61/18[8]	67/37(49)[6]	9.0[5]	2.08[5]	22.3[5]	0.65[6]	7.1[6]

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Number under initials of patients indicates period of maximum effect from Arfonad®.

† Pressures in parentheses are mean pressures.

‡ Averages are for five patients except where other numbers are shown in superscript parentheses.

§ Data are averages for ten subjects except as otherwise indicated by superscript numbers in parentheses.

Cardiac output and blood oxygen contents (Tables II to VII, Figures 3 and 4)

During the fall in vascular pressures, cardiac output increased slightly, 15 per cent, but nevertheless definitely and consistently (8 of 10 subjects) in both the normotensive and hypertensive subjects in congestive heart failure (Group I). The increase appears significant: p value for the 10 subjects lies between 0.05 and 0.02. The increases did not, however, bring the cardiac outputs to normal values. In contrast, cardiac output fell in four of the five noncardiac and compensated cardiac subjects (Group III) and remained unchanged in one. Statistically, the average fall of 18 per cent appears significant: p value between 0.05 and 0.02. The validity of these changes in cardiac output is indicated by the opposite, and again contrasting, changes in arterial-mixed venous oxygen difference, a better index of cardiac function than cardiac output in that the A-V oxygen difference relates the blood supply (cardiac output) to the need for it (oxygen consumption). Thus, in congestive heart failure (Group I), the A-V oxygen difference decreased by 22 per cent (p value significant, less than 0.01); but again the final values were not in the normal range. In contrast, A-V oxygen difference in the compensated cardiac and noncardiac subjects (Group III) changed variably (rose in three, decreased in one, unaltered in one) to give an average 5 per cent increase (p value a not significant 0.3). Furthermore, the decrease in A-V oxygen difference in congestive heart failure was associated with an average 5 to 10 per cent increase (p value between 0.1 and 0.05) in oxygen content of mixed venous blood. In contrast, an average 4 per cent decrease (p value between 0.1 and 0.05) in mixed venous oxygen content occurred in the noncardiac and compensated subjects. The changes in A-V oxygen difference were similar in normotensive and hypertensive subjects within a group.

As the period of vasodilatation progressed, the changes in cardiac output, A-V oxygen difference and oxygen content of mixed venous blood tended to lessen and revert toward control values in the subjects with congestive heart failure (Tables II and III) but were better maintained at their altered levels in the compensated cardiac and noncardiac

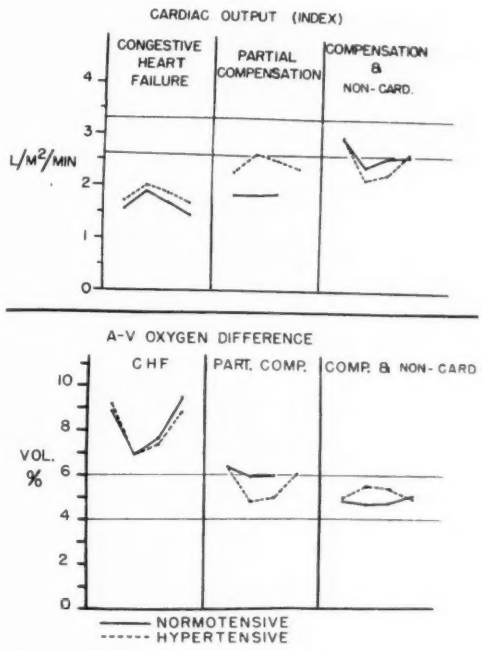

FIG. 4. EFFECT OF INTRAVENOUS INFUSION OF ARFONAD® ON CARDIAC OUTPUT AND A-V OXYGEN DIFFERENCE IN CONGESTIVE HEART FAILURE AND IN CARDIAC COMPENSATION

Details of plotting are the same as those in Figure 2.

subjects (Table V). After withdrawal of Arfonad® these functions returned to control values in the subjects with congestive failure but tended to remain at their altered values in the compensated cardiac and noncardiac subjects.

In the partially compensated subjects (Group II) the two hypertensive patients manifested changes similar to those in congestive heart failure; the two normotensive subjects developed no changes, nor did the two patients with "normal output" heart failure (Group IV).

In all subjects and at all levels of arterial oxygen content and arterial oxygen saturation, there occurred during vasodilatation a consistent fall (p value less than 0.01) in arterial oxygen content and oxygen saturation, amounting to 3 to 4 per cent in the noncardiac and compensated subjects and 6 to 8 per cent in the other three groups. These values returned well toward control levels after discontinuing the infusion of Arfonad®.

TABLE IV

Hemodynamic effects of Arfonad®: Partially compensated normotensive and hypertensive patients

Patient*		Cardiac index	A-V oxygen diff.	Arterial oxygen content	Arterial oxygen saturation	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure†	Systemic vascular resistance	Pulmonary "wedge" pressure	Right atrial pressure	Right ventricular pressure	Pulmonary artery pressure†	Pulmonary vascular resistance	Left ventricle		Right ventricle	
																	Minute work index	Stroke work index	Minute work index	Stroke work index
		L./M.²/min.	*Vol. %*	*Vol. %*	*%*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
										Normotensive patients										
J. J.	Avg. Cont.	1.75	6.96	19.38	96.2	12.42	120	6.55	90	133/88(108)	40.3		1							
(1)	Max. Vasod.	1.85	6.27	18.77	93.3	12.50	116	6.79	96	117/79(96)	34.1		−2							
	Avg. Vasod.	1.79	6.18	18.71	92.8	12.53	110	7.16	93	118/78(96)	35.2		−2							
	Post Vasod.																			
W.G.	Avg. Cont.	1.75	6.34	16.68	90.3	10.43	111	7.02	66	155/81(108)	37.6	14		33/11	32/14(19)	1.9	2.37	35.9	0.20	3.05
(1)	Max. Vasod.		4.33	15.00	80.9	10.67			65	111/54(75)					21/10(12)					
	Avg. Vasod.	2.70	5.43	15.61	84.2	10.17	158	8.92	56	103/51(70)	15.2	10		22/8	22/10(13)	0.7	2.33	41.7	0.19	3.47
	Post Vasod.	1.94	7.28	16.98	91.8	9.70	141	11.86	56	(103)	32.0	13		30/11	30/13(18)	1.7	2.51	44.9	0.20	3.49
										Hypertensive patients										
L. R.	Avg. Cont.	2.03	6.47	17.26	95.1	10.79	131	6.81	82	232/130(168)	44.7		5	71/8						
(1)	Max. Vasod.	2.16	4.87	16.80	92.6	11.93	105	6.13	68	166/93(119)	30.6		2							
	Avg. Vasod.	2.27	5.12	16.58	91.4	11.47	116	6.59	65	166/95(121)	29.7		2	48/7						
	Post Vasod.	2.04	6.51	17.37	95.7	10.86	133	7.12	71	201/121(184)	48.8		8							
S. T.	Avg. Cont.	2.50	6.20	13.89	88.9	7.69	155	8.73	99	204/108(140)	27.5	28		57/10	55/26(39)	2.3	4.03	40.7	1.04	10.5
(1)	Max. Vasod.	2.85	4.72	12.89	82.3	8.17	134	7.82	89	172/89(113)	20.1				36/19(23)	2.9	4.35	48.8	0.86	9.7
	Avg. Vasod.	2.70	4.75	13.00	83.1	8.25	128	8.32	84	168/86(110)	20.8	7		32/2	37/19(23)	3.1	4.01	47.7	0.82	9.7
	Post Vasod.	2.65	5.59	13.77	88.0	8.17	146	8.87	89	192/99(130)	24.8	19		49/6	50/28(33)	2.7	4.22	47.7	1.03	11.6
Avg.	Avg. Cont.	2.00	6.49	16.80	92.6	10.31	129	7.36(3)	84	181/102(131)	37.6	21(2)	3(2)	54/10(3)	44/20(29)(2)	2.1(3)	3.20(2)	38.3(2)	0.62(2)	6.8(2)
4	Max. Vasod.	2.28(3)	5.05	15.86	87.2	10.82	118(3)	6.92(2)	80	142/78(101)	28.3(2)		0(2)		29/14(18)(2)	2.9(1)	4.35(1)	48.8(1)	0.86(1)	9.7(1)
Pts.‡	Avg. Vasod.	2.25(3)	5.37	15.98	87.8	10.60	128	7.36(3)	74	138/77(100)	28.5(3)	8(2)	0(2)	34/5(3)	30/15(18)(2)	1.9(2)	3.17(2)	44.7(2)	0.50(2)	6.6(2)
	Post Vasod.	2.21(3)	6.46(3)	16.04(3)	91.8(3)	9.57(3)	140(3)	9.29(3)	72(3)	196/110(139)(3)	35.2(3)	16(2)	8(1)	40/8(2)	40/20(26)(2)	2.2(3)	3.37(2)	46.5(2)	0.61(2)	7.5(2)

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Numbers under initials of patients indicate period of maximum effect from Arfonad®.

† Pressures in parentheses are mean pressures.

‡ Averages are for four patients except as otherwise indicated by superscript numbers in parentheses.

TABLE V

Hemodynamic effects of Arfonad®: Noncardiac subjects and compensated cardiac patients

Patient*	Cardiac index	A-V oxygen diff.	Arterial oxygen content	Arterial oxygen saturation	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure†	Systemic vascular resistance	Pulmonary "wedge" pressure	Right atrial pressure	Right ventricular pressure	Pulmonary artery pressure†	Pulmonary vascular resistance	Left ventricle		Right ventricle	
																Minute work index	Stroke work index	Minute work index	Stroke work index
	L./M.²/min.	*Vol. %*	*Vol. %*	*%*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
Noncardiac control subjects																			
L. K. Avg. Cont.	2.99	4.89	15.30	92.3	10.41	146	6.81	85	134/81(103)	19.6	5		16/0	16/7(11)	1.2	4.22	49.6	0.47	5.6
(2) Max. Vasod.	2.67	4.90	14.20	85.6	9.30	131	6.61	100	104/71(85)	17.9			12/1	16/8(11)	2.4	3.26	32.7	0.38	3.8
Avg. Vasod.	2.67	4.99	14.43	87.0	9.43	134	6.65	100	108/71(85)	18.5	0		13/1	15/7(10)	2.1	3.26	32.7	0.35	3.5
Post Vasod.	2.78	4.96	14.36	86.6	9.40	138	6.95	88	130/80(100)	20.2	5		17/2	20/9(10)	1.0	3.80	43.2	0.32	3.6
J. H. Avg. Cont.	3.07	4.28	16.68	93.4	12.40	131	8.41	73	140/68(94)	17.4	5		20/2	19/5(10)	0.9	3.94	53.9	0.35	4.8
(2) Max. Vasod.	2.41	4.90	16.48	92.2	11.58	118	9.67	84	115/65(82)	19.4			13/−1	12/3(7)	0.7	2.71	32.2	0.28	3.3
Avg. Vasod.	2.32	4.82	16.48	92.2	11.66	112	9.03	83	116/66(84)	20.8	4		13/0	13/3(7)	0.7	2.67	32.2	0.23	2.8
Post Vasod.	2.54	5.24	16.66	93.3	11.42	134	9.40	72	166/80(110)	23.5	6	1	26/6	25/7(13)	1.6	3.80	52.8	0.26	3.6
Normotensive compensated cardiac patients																			
J. M. Avg. Cont.	2.72	4.94	13.78	93.2	8.83	134	6.40	70	141/74(98)	20.1			20/4	21/8(12)	0.6	3.49	49.8	0.31	4.5
(1) Max. Vasod.	2.54	4.76	13.56	91.7	8.80	120	7.38	81	110/63(78)	17.1			15/3	16/5(10)	1.4	2.70	33.4	0.26	3.2
Avg. Vasod.	2.69	4.50	13.20	89.2	8.70	120	7.57	78	113/63(80)	16.7	4		15/4	16/7(11)	1.5	2.94	37.8	0.27	3.5
Post Vasod.	2.49	5.11	13.58	91.9	8.47	147	8.87	63	153/78(108)	23.8	9	4	21/6	22/9(17)	1.8	3.54	56.3	0.40	6.3
Hypertensive compensated cardiac patients																			
A. H. Avg. Cont.	2.52	5.43	15.89	81.1	10.45	133	4.21	85	222/118(158)	43.1			47/7	48/16(27)				0.73	8.5
(2) Max. Vasod.	1.89	5.75	16.06	82.0	10.31	109	4.88	81	130/70(95)	34.7			31/4	33/12(20)				0.44	5.4
Avg. Vasod.	1.97	5.88	16.12	82.3	10.25	116	4.63	82	153/79(114)	39.9			32/6	36/13(21)				0.43	5.2
Post Vasod.	2.16	5.37	16.17	82.7	10.80	116	4.12	80	223/101(161)	51.1		2	48/7	53/16(28)				0.65	8.2
E. S. Avg. Cont.	3.36	4.54	13.54	91.8	9.01	153	7.88	76	263/104(161)	28.3	15		36/4	41/20(23)	1.4	7.06	92.9	0.92	12.1
(1) Max. Vasod.	2.51	5.03	12.35	83.5	7.32	127	7.92	63	195/82(112)	26.4			21/2	23/11(13)	2.2	3.90	61.9	0.40	6.3
Avg. Vasod.	2.52	4.93	12.41	83.9	7.48	125	8.00	67	196/80(115)	27.1	4		21/2	22/10(12)	1.9	4.03	60.1	0.36	5.4
Post Vasod.	3.09	4.54	12.96	87.7	8.42	141	8.39	74	257/100(153)	29.8	13	0	38/4	37/19(22)	1.8	6.23	84.2	0.80	10.8
Avg. Avg. Cont.	2.93	4.81	15.04	90.4	9.82	139	6.80	78	180/87(123)	25.7	8(3)		28/4	29/11(17)	1.0(4)	4.67(4)	61.6(4)	0.56	7.1
5 Max. Vasod.	2.40	5.07	14.53	87.0	9.46	121	7.29	82	131/70(91)	23.1			18/2	20/8(12)	1.6(4)	3.14(4)	40.1(4)	0.35	4.4
Pts.‡ Avg. Vasod.	2.43	5.02	14.50	86.9	9.50	121	7.18	82	137/72(95)	24.6	3(4)		19/3	21/8(12)	1.6(4)	3.23(4)	40.7(4)	0.33	4.1
Post Vasod.	2.61	5.04	14.75	88.4	9.70	135	7.54	75	186/87(126)	29.7	8(4)		30/5	31/12(20)(4)	1.6(4)	4.34(4)	59.1(4)	0.48	6.5

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Number under initials of patients indicates period of maximum effect from Arfonad®.

† Pressures in parentheses are mean pressures.

‡ Averages are for five patients except as otherwise indicated by superscript numbers in parentheses.

TABLE VI

Hemodynamic effects of Arfonad®: "Normal output" congestive heart failure

Patient*		Cardiac index	A-V oxygen diff.	Arterial oxygen content	Arterial oxygen saturation	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure†	Systemic vascular resistance	Pulmonary "wedge" pressure	Right atrial pressure	Right ventricular pressure	Pulmonary artery pressure†	Pulmonary vascular resistance	Left ventricle		Right ventricle	
																	Minute work index	Stroke work index	Minute work index	Stroke work index
		L./M.²/min.	*Vol. %*	*Vol. %*	*%*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
										Normotensive patient										
B. O.	Avg. Cont.	3.32	4.83	14.42	90.7	9.59	160	6.67	95	168/84(116)	24.8		14	60/20						
(2)	Max. Vasod.	3.90	5.06	12.61	79.1	7.55	196	9.70	87	115/55(75)	12.6		12	50/20						
	Avg. Vasod.	3.76	4.92	12.86	80.7	7.93	186	9.05	88	105/51(70)	12.4		12	50/18						
	Post Vasod.	2.38	5.46	13.17	82.7	7.71	130	6.17	92	156/71(93)	25.4		18	55/18						
										Hypertensive patient										
E. S.	Avg. Cont.	3.23	4.84	12.05	88.5	7.21	156	8.45	80	223/98(147)	27.3	23		42/2	46/23(29)	1.2	5.76	71.7	1.27	15.9
(1)	Max. Vasod.	2.88	4.78	11.65	85.3	6.87	137	8.69	76	170/75(105)	22.0			22/1	20/6(17)	1.9	4.02	52.9	0.67	8.8
	Avg. Vasod.	2.87	4.88	11.95	87.6	7.07	138	8.92	72	165/72(102)	21.7	8		22/1	23/9(16)	1.6	3.86	53.4	0.61	8.4
	Post Vasod.	2.72	5.60	12.72	93.5	7.12	152	9.78	80	220/94(135)	29.5	17		47/5	48/20(30)	2.9	4.63	57.9	0.98	12.2
Avg.	Avg. Cont.	3.28	4.84	13.24	89.6	8.40	158	7.56	88	196/91(132)	26.0			51/11						
2	Max. Vasod.	3.39	4.92	12.13	82.2	7.21	167	9.20	82	143/65(90)	17.3			36/10						
Pts.	Avg. Vasod.	3.32	4.90	12.40	84.2	7.50	162	8.99	80	135/62(86)	17.0			36/10						
	Post Vasod.	2.55	5.53	12.95	88.1	7.42	141	7.98	86	188/82(114)	27.4			51/12						

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Number under initials of patient indicates period of maximum effect of Arfonad®.

† Pressures in parentheses are mean pressures.

TABLE VII

Hemodynamic effects of Arfonad®: Percentage changes from average of control values *

Periods†	Cardiac index	A-V oxygen diff.	Arterial oxygen content	Mixed venous oxygen content	Oxygen consumption	Ventilation S.T.P.	Heart rate	Systemic arterial pressure (mean)	Systemic vascular resistance	Pulmonary "wedge" pressure	Right ventricular pressure	Pulmonary artery pressure (mean)	Pulmonary vascular resistance	Left ventricle		Right ventricle	
														Minute work index	Stroke work index	Minute work index	Stroke work index
	L./M.²/min.	*Vol. %*	*Vol. %*	*Vol. %*	*ml./M.²/min.*	*L./min.*	*per min.*	*mm. Hg*	*mm. Hg/L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg/L./min.*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*	*Kg. M./M.²/min.*	*Gm. M./M.²/beat*
Group I. Congestive heart failure (10 patients)																	
Avg. Cont.–Max. Vasod.	+17	−22	−7	+10	−12	− 6	−11	−24	−35		−26/−33	−21	− 30	−11	+ 1	− 5	+ 9
Avg. Cont.–Avg. Vasod.	+ 9	−15	−6	+ 4	−11	− 4	−10	−22	−29	−24	−21/−22	−17	− 19	− 8	− 3	−10	+ 1
Avg. Cont.–Post-Vasod.	− 4	+ 2	−3	− 8	− 4	+ 6	−10	− 5	− 2	− 7	0/0	+ 2	− 2	−13	− 7	− 4	+ 3
Group II. Partial compensation (4 patients)																	
Avg. Cont.–Max. Vasod.	+14	−22	−6	+ 5	− 6	− 6	− 5	−23	−25			−38	+ 27	+ 8	+20	−17	− 8
Avg. Cont.–Avg. Vasod.	+12	−17	−5	+ 3	− 6	0	−12	−24	−24	−62	−37/−50	−38	− 13	− 1	+16	−13	+ 3
Avg. Cont.–Post-Vasod.	+10	0	−4	− 7	+10	+26	−14	+ 6	− 6	−24	−26/−29	−10	+ 5	+27	+21	− 2	+12
Group III. Noncardiac control and compensation (5 patients)																	
Avg. Cont.–Max. Vasod.	−18	+ 5	−3	− 4	−12	+ 7	+ 5	−26	−10		−36/−50	−29	+ 61	−30	−35	−31	−35
Avg. Cont.–Avg. Vasod.	−17	+ 4	−4	− 3	−13	+ 6	+ 5	−23	− 4	−62	−32/−25	−29	+ 59	−28	−33	−35	−39
Avg. Cont.–Post-Vasod.	−11	+ 5	−2	− 1	− 3	+11	− 4	+ 2	+16	0	+ 7/+25	+18	+ 68	− 6	− 3	−11	− 7
Group IV. "Normal output" congestive heart failure (2 patients)																	
Avg. Cont.–Max. Vasod.	+ 3	+ 2	−8	−14	+ 3	+22	− 7	−32	−33		−29/−9	−41	+ 58	−30	−26	−47	−44
Avg. Cont.–Avg. Vasod.	+ 1	+ 1	−6	−11	0	+19	− 9	−35	−35	−65	−29/−9	−45	+ 33	−33	−26	−52	−47
Avg. Cont.–Post-Vasod.	−22	+14	−2	−12	−10	+ 6	− 2	−14	+ 5	−26	0/+9	+ 3	+142	−20	−19	−23	−23

* The data are averaged data for the patients in each group and are percentage values to the nearest whole number.

† The analysis represents, for each group, the percentage change from the average of the control values (avg. cont.) as follows: *first row*, period of maximum effect (max. vasod.); *second row*, average of all vasodilatation periods (avg. vasod.); *third row*, postvasodilatation period (post-vasod.).

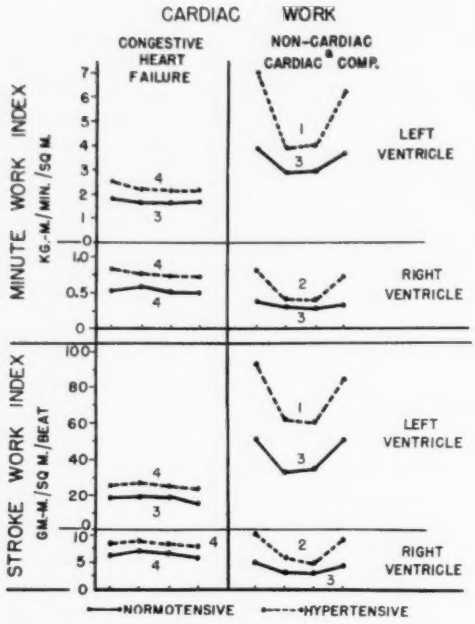

FIG. 5. EFFECT OF INTRAVENOUS INFUSION OF ARFONAD® ON CARDIAC WORK IN CONGESTIVE HEART FAILURE AND CARDIAC COMPENSATION

Details of plotting are the same as in Figure 2, except that the horizontal line separates the data for the two ventricles and not the range of normal values. The numbers indicate subjects averaged for the adjacent lines.

Cardiac work (Tables II to VII, Figure 5)

In the patients with congestive heart failure (Group I) minute work index and stroke work index of both the left and right ventricle remained essentially unchanged, or perhaps decreased slightly, during the fall in vascular pressures and rise in cardiac output which accompanied vasodilatation (Tables II, III and VII). The lower values tended to persist in the postvasodilatation period. In contrast, in the compensated cardiac and noncardiac subjects (Group III) both left and right ventricular minute work index and stroke work index decreased, by 30 to 40 per cent, during the vasodilatation and tended to return to control values in the postvasodilatation period (Tables V and VII). Hypertensive and normotensive subjects in congestive heart failure had largely similar and equal responses (Figure 5). In the compensated cardiac and noncardiac subjects, the decreases in both cardiac work indices were greater in hypertensive than in normotensive subjects. Because of the small numbers of subjects in the several groups, statistical analysis of the data on cardiac work did not prove helpful.

In the one subject (hypertensive) with partial compensation (Group II) vasodilatation resulted in increases in minute work index (15 per cent) and stroke work index (25 per cent) of the left ventricle and decreases in both minute work index (15 per cent) and stroke work index (5 per cent) of the right ventricle (Table IV). The one (hypertensive) subject with "normal output" congestive failure (Group IV) developed marked decreases in both minute work and stroke work indices of the left (30 per cent) and right (45 per cent) ventricles (Table VI), a decided contrast with the results in low-output congestive heart failure.

Renal functions and water and electrolyte excretions (Tables VIII to X, Figure 6)

The changes in renal hemodynamic functions during vasodilatation were relatively small and variable. Glomerular filtration rate altered slightly and inconsistently in all groups, with decreases perhaps more frequent than increases. In subjects with congestive heart failure (Group I) renal plasma flow tended to increase (20 per

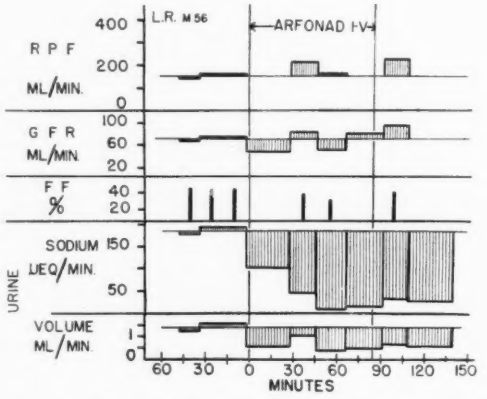

FIG. 6. TYPICAL SERIAL DETERMINATIONS OF RENAL HEMODYNAMICS AND SODIUM AND WATER EXCRETIONS IN A REPRESENTATIVE SUBJECT (L.R.) WITH CONGESTIVE HEART FAILURE

Rate of intravenous infusion of Arfonad®, 0.8 mg. per minute. RPF designates renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction.

TABLE VIII

Effects of Arfonad® on renal hemodynamics and urinary excretions: Congestive heart failure

Patient*		Glomerular filtration rate	Renal plasma flow	Filtration fraction	Renal blood flow	Renal fraction of cardiac output	Renal vascular resistance	Urine output	Urinary sodium excretion	Urinary potassium excretion
		ml./min.	*ml./min.*	*%*	*ml./min.*	*%*	*mm. Hg L./min.*	*ml./min.*	*μEq./min.*	*μEq./min.*
					Normotensive patients					
G. L. (1)	Avg. Cont.		165		262	9.9	376	0.4	<0.2	26
	Max. Vasod.		214		340	11.5	176	0.7	<0.2	34
	Avg. Vasod.		156		248	10.3	284	0.5	<0.2	26
	Post Vasod.		203		322	15.4	273	0.6	<0.2	38
B. K. (1)	Avg. Cont.	54	162	33	337	11.9	289	0.9	5	53
	Max Vasod.	51	180	28	375	9.5	200	0.6	1	46
	Avg. Vasod.	53	184	28	384	11.7	207	0.8	2	54
	Post Vasod.									
					Hypertensive patients					
L. R. (2)	Avg. Cont.	71	156	46				1.4	187	73
	Max. Vasod.	51	162	32				0.3	<10	37
	Avg. Vasod.	76	200	37				0.7	30	50
	Post Vasod.							0.6	29	45
R. H. (2)	Avg. Cont.		264					1.1	101	
	Max. Vasod.		381					1.1	144	
	Avg. Vasod.		328					0.8	115	
	Post Vasod.		254					0.8	102	
B. K. (1)	Avg. Cont.	60	125	46	223	8.6	430	0.9	15	5
	Max. Vasod.	76	157	48	280	14.4	243	0.8	7	5
	Avg. Vasod.	74	138	54	247	11.7	320	0.9	4	5
	Post Vasod.	69	131	52	234	9.1	355	0.9	8	5
S. T. (2)	Avg. Cont.	17	59	29	77	1.6	1,562	2.0	187	28
	Max. Vasod.	14	53	26	72	1.2	1,194	1.1	83	26
	Avg. Vasod.	13	54	23	74	1.3	1,228	1.1	85	30
	Post Vasod.	20	60	34	82	2.0	1,366	1.7	134	44
Avg. 6 Pts.†	Avg. Cont.	50[4]	155	38[4]	225[4]	8.0[4]	664[4]	1.1	82	37[5]
	Max. Vasod.	48[4]	191	33[4]	267[4]	9.2[4]	453[4]	0.8	41	30[5]
	Avg. Vasod.	54[4]	177	36[4]	238[4]	8.8[4]	510[4]	0.8	46	33[5]
	Post Vasod.	45[2]	162[4]	43[2]	213[3]	8.9[3]	664[3]	0.9[5]	73[5]	33[4]
					"Normal output" congestive heart failure					
B. O. (1)	Avg. Cont.	56	162	34	249	6.1	410	5.6	867	<30
	Max. Vasod.	35	138	25	212	4.7	259	0.4	65	16
	Avg. Vasod.	40	162	25	248	5.3	236	0.7	106	26
	Post Vasod.	50	165	30	254	8.6	295	3.0	514	48

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Number under initials of patients indicates period of maximum effect of Arfonad®.

† Averages are for six patients except as otherwise indicated by superscript numbers in parentheses.

cent) and similar, if slightly less marked, rises occurred in renal blood flow and in the renal fraction (Tables VIII and X). No similar changes occurred in the other three groups of subjects, where decreases in these three renal hemodynamic functions were more common than increases. However, consistent decreases in filtration fraction, 10 to 15 per cent, and in renal vascular resistance, 15 to 30 per cent, occurred in all four groups of subjects and to approximately the same extent. In the few patients studied no difference was noted between the normotensive and hypertensive subjects.

In all four groups the renal excretion of water and sodium, and less markedly and regularly of potassium, decreased during the period of vasodilatation, usually by about 30 per cent, but by as much as 80 to 90 per cent for water and sodium when their initial excretions were large. The decrease in urine volume was usually associ-

ated with a decrease in glomerular filtration but falls in electrolyte excretion occurred without appreciable changes in glomeruluar filtration. The water and electrolyte excretions remained decreased, at least to some degree, during the post-vasodilatation period, when cardiovascular dynamics had already returned to, or well toward, control values.

DISCUSSION

On the whole the patients, even with severe congestive heart failure, tolerated surprisingly well the irksome and long procedures imposed by this study. The complexity of the procedures, the variations in circulatory state between patients even of the same group, and the difficulty of obtaining a similar circulatory state or change of state during vasodilatation probably contributed to the variability of the data obtained. This variability and the relatively few subjects studied do not permit strict quantitative relationships to be drawn between the various functions. Nevertheless, the qualitative changes which emerged appear valid.

Nature of the cardiovascular and renal responses during vasodilatation

The present observations substantiate previously reported studies, usually of individual or few functions, which indicate that primary vasodilatation, induced by various vasodilating agents, produces changes similar to those here observed: decreases in pressure in all parts of the compensated circulatory system in both normal and hypertensive circulations (23–26) and in congestive heart failure (23–25, 27–29), a rise in cardiac output in congestive heart failure (23–25, 27) but a fall in output in the normal circulations (23, 25, 26) and a decrease in water and electrolyte excretions, associated with inconsistent changes in renal hemodynamics, in all circulatory states (23, 25, 26, 30, 31). Through repeated, integrated and simultaneous determinations of multiple functions, the present observations permit a more direct correlation of the changes in the several functions.

One question in the present study was to examine whether differences in vascular tonus between normal and congested circulations might be disclosed by the need for different amounts of

TABLE IX

Effects of Arfonad® on renal hemodynamics and urinary excretions: Noncardiac subjects and partially compensated cardiac patients

Patient*		Glomerular filtration rate	Renal plasma flow	Filtration fraction	Renal blood flow	Renal fraction of cardiac output	Renal vascular resistance	Urine output	Urinary sodium excretion	Urinary potassium excretion
		ml./min.	*ml./min.*	%	*ml./min.*	%	*mm. Hg/L./min.*	*ml./min.*	*μEq./min.*	*μEq./min.*
Noncardiac control subject										
L. K.	Avg. Cont.	102	539	19	842	16.1	124	0.5	134	41
(1)	Max. Vasod.	100	584	17	885	21.2	106	0.4	60	46
	Avg. Vasod.	104	571	18	866	18.4	99	0.4	64	45
	Post Vasod.	113	581	19	881	18.1	111	0.6	30	33
Normotensive partially compensated cardiac patient										
J. J.	Avg. Cont.	67	209	32	362	13.5	299	1.2	83	61
(1)	Max. Vasod.	61	196	31	356	12.6	275	1.0	73	51
	Avg. Vasod.	66	214	31	390	14.2	252	1.1	76	56
	Post Vasod.									
Hypertensive partially compensated cardiac patient										
L. R.	Avg. Cont.	101	251	41				6.3	619	79
(1)	Max. Vasod.	56	157	36				0.4	28	43
	Avg. Vasod.	70	211	34				0.4	19	56
	Post Vasod.							1.1	74	80

* For each subject the data are: *first row*, average of all control periods; *second row*, period of maximum effect; *third row*, average of all vasodilatation periods; *fourth row*, postvasodilatation period. Number under initials of patients indicates period of maximum effect of Arfonad®.

TABLE X

Renal hemodynamic and urinary effects of Arfonad®: Percentage changes from average control values *

Periods†	Glomerular filtration rate	Renal plasma flow	Filtration fraction	Renal blood flow	Renal fraction of cardiac output	Renal vascular resistance	Urine output	Urinary sodium excretion	Urinary potassium excretion
	ml./min.	*ml./min.*	%	*ml./min.*	%	*mm. Hg/L./min.*	*ml./min.*	*μEq./min.*	*μEq./min.*
Group I. Congestive heart failure (6 patients)									
Avg. Cont.–Max. Vasod.	− 4	+23	−13	+19	+15	−32	−31	−50	−19
Avg. Cont.–Avg. Vasod.	+ 8	+14	− 5	+ 6	+10	−23	−32	−44	−11
Avg. Cont.–Post-Vasod.	−10	+ 4	+13	− 5	+11	0	−20	−11	−11
Group II. Partial compensation (2 patients)									
Avg. Cont.–Max. Vasod.	−30	−23	− 6	− 2	− 7	− 8	−81	−86	−33
Avg. Cont.–Avg. Vasod.	−19	− 8	−11	+ 8	+ 5	−16	−80	−86	−20
Avg. Cont.–Post-Vasod.							−71	−79	+14
Group III. Noncardiac control and compensation (1 patient)									
Avg. Cont.–Max. Vasod.	− 2	+ 8	−10	+ 5	+32	−14	−30	−55	+12
Avg. Cont.–Avg. Vasod.	+ 4	+ 6	− 5	+ 3	+14	−20	−26	−52	+10
Avg. Cont.–Post-Vasod.	+13	+ 8	0	+ 5	+12	−10	+13	−78	−19
Group IV. "Normal output" congestive heart failure (1 patient)									
Avg. Cont.–Max. Vasod.	−38	−15	−26	−15	−23	−37	−93	−92	
Avg. Cont.–Avg. Vasod.	−29	0	−26	0	−13	−42	−88	−88	
Avg. Cont.–Post-Vasod.	−11	+ 2	−12	+ 2	+41	−28	−47	−41	

* The data are averaged data for the patients in each group and are percentage values to the nearest whole number.
† The analysis represents, for each group, the percentage change from the average of the control values (avg. cont.) as follows: *first row*, period of maximum effect (max. vasod.); *second row*, average of all vasodilatation periods (avg. vasod.); *third row*, postvasodilatation period (post-vasod.).

vasodilator agent in the two states. The data do not provide an answer. The two noncardiac subjects required the largest amounts of Arfonad® used. The same amount of Arfonad® appeared to produce larger actual falls in vascular pressures in congestive heart failure but the percentile changes in pressure were as great, and even greater, in the normal circulations, particularly in regard to residual or filling pressures.

The fall in systemic and renal vascular resistances during both rising and falling cardiac outputs and in both the normal circulation and in congestive heart failure indicates that Arfonad® produced primary vasodilatation of the systemic vascular circuit. A similar fall in pulmonary vascular resistance in congestive heart failure suggests a primary dilatation of the pulmonary vasculature and not solely a redistribution of blood from the pulmonary vascular bed into the concomitantly dilating systemic vessels (32). Arfonad® is considered to be primarily a ganglionic blocking agent and the decreases in vascular resistances may, at first, be taken as evidence that the vasodilatations are of neurogenic origin. However, a direct dilator action of Arfonad® on blood vessels has been reported (13). The data cannot, therefore, resolve the question of the existence of sympathetic innervation of the pulmonary vessels. This is particularly so since pulmonary vascular resistance actually rose in the more normal circulations (Group III), presumably in response to the distribution of blood away from the lung into the systemic vascular bed.

No explanation is apparent for the unexpected fall in oxygen consumption during vasodilatation, unless the drug actually lowers the metabolic rate. It is also possible that tissue metabolic rate did not change and that the observed low pulmonary uptake of oxygen was supplemented by additional and unaccounted oxygen from other sources. No such sources are apparent.

The consistently lowered arterial oxygen content during vasodilatation was associated with no significant change in respiratory rate or minute volume of respiration. Accordingly, it is unlikely that impaired alveolar ventilation with decreased alveolar oxygen tension was responsible for the arterial unsaturation. An intriguing possible ex-

planation might be the opening up during vasodilatation of communications between pulmonary arterial and pulmonary venous channels, by passing the alveoli and permitting a greater degree of venous admixture.

Since hypoxia has been shown to induce hemodynamic effects, the fall in arterial oxygen saturation during vasodilatation introduces a complicating factor in the analysis of the results obtained. In noncardiac subjects, arterial oxygen saturation down to 80 or 82 per cent is tolerated without change in cardiac output, while hypoxia of greater degree causes an increase in cardiac output (33–35). In the present 21 observations arterial oxygen saturation during vasodilatation fell to 82 per cent or below only six times; 12 times the saturation remained above 85 per cent and three times above 90 per cent. It appears unlikely that this degree of hypoxia influenced the cardiac output in the noncardiac and compensated cardiac subjects. Two further points support this conclusion: The change in cardiac output during the hypoxia was opposite (a fall) to the expected increases induced by significant hypoxia and the degree of change in cardiac output was not related to the extent of fall in arterial oxygen saturation. Data indicating the effect of hypoxia on cardiac function of patients with congestive heart failure were not located. In the present study the increase in cardiac output during vasodilatation in congestive failure patients is considered to be unaffected by the associated hypoxia, largely for the reason already cited, but particularly because the induced hypoxia was rarely down to 80 per cent arterial oxygen saturation and because the changes in cardiac output were much the same whether arterial oxygen saturation fell considerably (to 82 per cent) or only minimally (to 88 per cent).

Comparison of effects produced by vasodilatation with effects produced by digitalis

In congestive heart failure, some of the effects produced by intravenously administered Arfonad® were strikingly similar to the effects which followed intravenous administration of digitalis glycosides (9, 21). In each instance there occurred: rapid onset of effect, fall in intracardiac pressures, decrease in vascular resistances, some decrease in heart rate, increase in cardiac output, decrease in A-V oxygen difference, slight decrease in oxygen consumption and slight decrease in arterial oxygen content and saturation. Single therapeutic doses of the glycosides generally produced more marked effects than those here evoked by primary vasodilatation, but in individual instances Arfonad® produced just as marked results as digoxin. There were, however, important differences in the results produced by the two drugs. The hemodynamic effects produced by the digitalis glycoside were sustained, whereas the responses induced by the vasodilator drug did not outlast its infusion and even tended to decrease toward the later part of the infusion. Digoxin did not lower systemic arterial pressure and usually increased it slightly. Accordingly, during the action of digoxin the lowering of intracardiac filling pressures was associated with increased cardiac minute and stroke work, in contrast to the unchanged cardiac work noted during the vasodilator action of Arfonad®. The hemodynamic effects produced by digitalis glycosides were, therefore, consistent with Starling's law; the effects produced by Arfonad® were perhaps not. The differences in cardiac work produced by the two drugs permit speculation regarding a possible difference in primary action of the drugs in congested failing circulations: the glycosides affecting heart muscle directly to increase myocardial action and produce greater work, the vasodilator agent apparently having no direct myocardial effect but permitting the unaltered heart muscle to eject more blood against the lowered vascular resistance.

The renal effects of the two drugs were in striking contrast: A diuresis of water and electrolytes followed administration of digoxin, whereas Arfonad® produced a reduction in water and electrolyte excretion. This difference in urinary excretions occurred even though both drugs induced similar slight, but inconsistent, alterations in renal hemodynamic functions. In view of the reported decreased urinary excretion of water and electrolytes when renal venous pressure is raised (36–38), a reduction of elevated renal venous pressure, such as probably was produced by Arfonad®, would be expected to produce an increased renal excretion of water and electrolytes—as was the case with digoxin. The falls in systemic arterial pressure were not so great as to jeopar-

dize glomerular filtration pressure. Furthermore, in animal experiments, diureses have been reported following removal of sympathetic innervation of the kidney (39). In view of these related findings the absence of a diuresis following Arfonad® is contrary to expectation and remains unexplained.

Cardiovascular changes during vasodilatation and Starling's law

Although the circulation, as measured by cardiac output and A-V oxygen difference, improved when the high intracardiac filling pressures in congestive heart failure were lowered, and became impaired, in the majority of instances, when the normal ventricular filling pressures of noncardiac and compensated cardiac subjects were decreased, these changes are not evidence that Starling's law was fulfilled (40). Moreover, there was a discordant observation: Often in congestive heart failure the increase in cardiac output, which occurred when the elevated filling pressures were first lowered, tended not to persist and cardiac output gradually decreased even though ventricular filling pressures remained at their lowered levels.

When Starling's law is considered in its correct terms of cardiac work, it appears that cardiac work did not increase when the elevated intracardiac filling pressures of congestive heart failure were lowered. In both normotensive and hypertensive patients with congestive heart failure, minute work index and stroke work index of both the left and right ventricles remained essentially unchanged as ventricular filling pressures fell. In the normotensive and hypertensive compensated cardiac patients and the noncardiac subjects the fall in intracardiac filling pressures was accompanied by the expected decreases in minute work index and stroke work index of both the left and right ventricles.

The interpretation of these changes in heart work in reference to Starling's law is rendered difficult, perhaps impossible, by the occurrence in the present observations of the fall in arterial pressures, both systemic and pulmonic. Since cardiac work is a function of both cardiac output and the arterial pressure against which the output is ejected, a reduction in arterial pressures represents an influence toward decreased cardiac work. Thus, in the patients with congestive heart failure, the tendency for cardiac work to increase as a result of the greater cardiac output was offset by the lowering of arterial pressures and cardiac work remained essentially unchanged. In the compensated cardiac and noncardiac subjects the lowered arterial pressures had a greater influence in reducing the cardiac work than the observed small decreases in cardiac output.

Since the present experimental procedures lowered the pressures on both sides of the ventricles, it was not possible to determine the effect of changes in intracardiac filling pressures *alone*, as a test of Starling's law requires. However, if one accepts the work equation as a correct representation of the work performed by the heart and accordingly accepts the basic concept that work as calculated is equivalent irrespective of the differences in the individual components entering into work, then the present conditions of concomitantly lowered arterial pressures offer no bar to the application of a general principle, and hence of Starling's law. This argument would also accept as no bar the consideration that for the same calculated work, the heart tolerates (*i.e.*, lower myocardial oxygen consumption and energy expenditure for the same amount of external work) flow work better than pressure work. Accepting these basic concepts, then the present observations suggest that Starling's law applies when ventricular filling pressures are normal but not when ventricular filling pressures are elevated. An alternative interpretation would stress the decidedly different *changes* in cardiac work in the two circulatory states, in relation to each other. Thus, the unchanged cardiac work during the lowering of the elevated filling pressures in congestive heart failure represents a better cardiac performance (a relative increase in work) than the decreased cardiac work associated with the fall in the normal ventricular filling pressures of the normal circulation. Such an interpretation suggests that Starling's law applies in the failing as well as in the nonfailing circulations. However, this intepretation requires that the measurements in the patients with congestive heart failure were made at the flat portion of the cardiac work-filling pressure curve, where no change in work oc-

curs upon lowering of the filling pressure. It also requires that all 10 patients with congestive heart failure had cardiac functions of similar degrees of insufficiency—even when the degrees of congestive failure, as clinically evaluated, varied from relatively mild to very severe. Finally, this interpretation is not entirely consistent with the observed increase in cardiac output and work which occurs when patients with congestive heart failure recover compensation (*22*).

It is apparent that the observations in both the failing and nonfailing circulation *can* be interpreted as consistent with Starling's law, but in the failing circulations only if certain assumptions are made regarding the state of the congestive failure in the clinical material. It is apparent, too, that the whole issue requires observations made under conditions of unchanging arterial pressures.

"Beneficial" effect of peripheral vasodilatation in congestive heart failure

The observation that primary vasodilatation in congestive heart failure results in an increased blood flow (cardiac output) without an increase in cardiac work suggests clinical and therapeutic applications (41). Presumably lowering the pressure against which the heart must eject blood permits an impaired myocardium to circulate more blood without an intrinsic improvement in its contractile activity. Such a result can be considered of advantage to the circulation in that the supply of blood to the various organs can be increased, even when the failing myocardium is not capable of improvement and greater work. However, the present observations deal with short periods of time (one hour) and in some instances the increased cardiac output was not sustained for the entire interval of lowered vascular pressures. Accordingly, no extrapolation of the acute effects here observed to chronic vasodilatation in congestive heart failure is intended. Furthermore, should the acute decrease in urinary excretions, here produced by Arfonad®, persist during chronic vasodilatation, the long term effect of this vasodilator agent could be undesirable.

SUMMARY

1) Primary peripheral vasodilatation for one hour was produced by the intravenous infusion of the ganglionic blocking agent Arfonad® in 12 cardiac subjects during congestive heart failure, in seven cardiac subjects after recovery of compensation and in two noncardiac subjects.

2) In all subjects vascular and intracardiac pressures were lowered, by 20 to 30 per cent, in both the systemic and pulmonic circulations.

3) In 8 of 10 subjects with low output congestive heart failure the lowering of vascular pressures was associated with an increase in cardiac output of approximately 15 per cent. Since the oxygen content of mixed venous blood rose and A-V oxygen difference fell, the increase in cardiac output was considered a valid one.

4) In four of five noncardiac and compensated cardiac subjects the lowering of vascular pressures resulted in a decrease in cardiac output (approximately 15 per cent), a fall in oxygen content of mixed venous blood and a small rise in A-V oxygen difference.

5) In all groups of subjects peripheral vasodilatation was accompanied by consistent decreases in the excretion of water and sodium, while renal hemodynamic functions changed variably and slightly.

6) The effects were similar in hypertensive and normotensive subjects.

7) Because of the fall in arterial pressures against which the heart ejected blood, the increase in cardiac output during vasodilatation in the patients in congestive heart failure was achieved without increase in cardiac minute or stroke work. In the noncardiac and compensated cardiac subjects minute and stroke work of the heart decreased during vasodilatation and this decrease was due more to the fall in arterial pressure than to the decrease in cardiac output.

8) The fall in arterial pressures in the present observations makes it difficult, if not impossible, to determine whether Starling's law of the heart applies to the hemodynamic effects produced by peripheral vasodilatation. If one accepts the condition of altered arterial pressures, then Starling's law may be considered to have held in the noncardiac and compensated cardiac circulations but not in congestive heart failure, unless one assumes that cardiac function in all of these patients was on the flat portion of the cardiac work-filling pressure curve.

9) Arfonad® given intravenously in congestive

heart failure produced effects similar to digitalis glycosides with respect to decreased intracardiac pressures, increased cardiac output and decreased A-V oxygen difference. However, following digoxin, cardiac work increased and a diuresis of water and electrolytes was produced, whereas after Arfonad®, cardiac work remained unaltered and water and electrolyte excretions decreased.

10) It is not clear whether the increase in blood flow without increase in cardiac work, which follows peripheral vasodilatation by Arfonad®, is of benefit to the circulation in those states where the heart is so impaired that myocardial activity cannot be intrinsically improved.

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IMMUNOCHEMICAL STUDIES OF HUMAN SERUM GAMMA GLOBULINS *

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The establishment of the quantitative precipitin reaction as an analytical-chemical procedure by Heidelberger and Kendall (1) was followed in 1937 by Kendall's original use of the method in the estimation of human serum proteins in health and disease (2, 3). A major drawback to the use of this immunochemical method for obtaining meaningful human serum protein values has concerned the purity or homogeneity of the specific protein used as the antigen (4). In particular, the heterogeneity of the human gamma globulin fraction, as defined electrophoretically, has been pointed out (5). Cohn, Deutsch and Wetter have demonstrated distinctive immunochemical behavior in various subfractions of the human serum gamma globulins (6). In these studies, however, the principal differences in immunochemical behavior of the subfractions were noted in zones of antigen excess; this zone is not employed in the studies reported herein.

Although numerous physical, chemical and immunological criteria have been set forth for the establishment of protein purity, the lack of information regarding protein structure does not permit a clear definition of a single human protein species. In general, the proteins comprising the human serum gamma globulins separated by ethanol fractionation have an isoelectric distribution of pH 5.7 to 7.5; their electrophoretic mobilities range from 0.97 to 2.64. The marked antigenic similarity, indeed almost antigenic identity, of the members of this family of proteins in the zone of antibody excess, has been well documented (7, 8). For the purposes of this study it was felt that a physically or chemically homogeneous gamma globulin preparation, possessing immunologic homogeneity in the zone of antibody excess, might be employed for preparation of antibody to be used in a quantitative precipitin test for estimation of gamma-reactive globulin in human fluids. Because of the inadequacy of other methods, it was considered likely that this technique might be especially useful when the concentration of the gamma globulin to be measured is very low.

The physical, chemical and immunological similarities of the human gamma globulins and most specific antibodies are well documented (9, 10). On the biological level, the clinical entity agammaglobulinemia provides strong evidence in support of the concept that gamma globulin and most antibodies arise from a common source (11, 12). Accordingly, immunochemical estimations, patterned directly after the methods of Heidelberger and Kendall (1), have been applied to the study of agammaglobulinemic and normal human sera, and reference comparisons to electrophoretic observations have been accomplished. The purposes of the present study were several-fold and included the following: *1.* To establish the efficacy of the quantitative precipitin reaction for the accurate estimation of human serum gamma globulins. *2.* To measure the serum concentrations of gamma-reactive globulin in normal humans. *3.* To describe the quantitative gamma globulin deficit in patients with both congenital and acquired agammaglobulinemia. *4.* To establish the biological survival time of parenterally administered gamma globulin in humans by immunochemical means. *5.* To study the appearance of gamma-reactive globulins in the newborn baby of a mother with acquired agammaglobulinemia, and to relate the appearance of gamma globulin

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to the time of onset of specific immune globulin synthesis and release; to study the disposition of maternally transmitted gamma globulin by the agammaglobulinemic offspring of an immunologically normal female who had previously borne a son afflicted with the congenital form of the disease. 6. To compare maternal and cord serum concentrations of gamma-reactive globulin.

MATERIALS AND METHODS

Sixty immunologically normal children ranging in age from one day to 15 years: These children were patients at the University of Minnesota Hospitals, Minneapolis General Hospital and the Ancker Hospital of St. Paul, who had been admitted for study or treatment of congenital anomalies or of trauma. None had been troubled with recurrent infections, and in no case was there reason to suspect an abnormality of protein synthesis or an immunological deficit. Twenty-four immunologically normal, healthy adults: These subjects were laboratory and hospital personnel, ranging in age from 19 to 34 years. Thirty-one maternal cord serum pairs: In all cases, the maternal blood was drawn within two hours of parturition. Sera from 16 patients with agammaglobulinemia: Nine of these were patients with the congenital form of the disease, and seven with the acquired form. The subjects chosen for study of gamma globulin half-life determinations included four congenitally agammaglobulinemic patients and a normal newborn who was bled frequently during the neonatal period. All subjects were free of clinical infections at the time these studies were performed.

After preinjection bleedings, each subject in the half-life study was given gamma globulin in various dosages intramuscularly. Venous blood was drawn thereafter at varying intervals. The bloods were allowed to clot at room temperatures for one hour, placed at 2° C. overnight, the sera separated by centrifugation in the cold, quick-frozen at −70° C., and stored at −26° C. until ready for use.

The decline of maternally transmitted gamma globulin was assessed by studying the male offspring of an apparently normal mother who had previously borne a congenitally agammaglobulinemic male. In this study, blood samples were drawn from the jugular vein of the baby in the neonatal period and at intervals throughout the first year.

The appearance of gamma globulin in the newborn and its quantitative characteristics during the first year of life were determined in the unique situation of a normal female offspring of a patient with acquired agammaglobulinemia, reported upon in detail elsewhere (13, 14). Frequent venous blood samples were obtained from the baby's jugular vein throughout the first year of life.

Total serum proteins were determined by the biuret method of Weichselbaum (15). Quantitative precipitin determinations for human gamma-reactive globulin were performed as outlined by Kabat and Mayer (16). A highly purified gamma globulin preparation obtained by ethanol fractionation was kindly supplied by Dr. J. T. Edsall, and served as antigen in these studies. The method of Kendall (2) for alum precipitation of the antigen and that of Jager, Smith, Nickerson and Brown (17) for the immunization schedule were adopted. Anti-human gamma globulin was produced in albino mixed hybrid rabbits weighing 4 to 5 Kg. The alum precipitated antigen was administered intravenously only and in a concentration of 1 mg. per ml. over a three week period. Each animal received a total of 12 mg. of the alum precipitated material. On the eighth day following the last injection of antigen, each animal was exsanguinated by cardiac puncture under sterile conditions. The bloods obtained were allowed to clot at room temperatures for one hour, placed at 2° C. overnight, the clots then rimmed and the tubes centrifuged at 1,500 rpm for 20 minutes in an International PR-1 centrifuge; the sera were decanted and pooled after preliminary testing for antibody content. The pool was then recentrifuged at 2,000 rpm for 20 minutes, decanted, and merthiolate was added to a final concentration of 1 part in 10,000. The pool was then heated at 56° C. for 30 minutes, allowed to come to room temperature, dispensed into sterile glass tubes, quick-frozen in an alcohol-acetone dry ice bath, and stored at −26° C. until ready for study. Prior to use in these and all subsequent determinations, sera and antisera were clarified by centrifugation for one hour in the cold at 3,000 rpm.

Absorption-precipitin study. A solution of the gamma globulin was prepared in nitrogen-free diluent, consisting of 0.01 molar phosphate at pH 7.4, and brought to ionic strength 0.145 with sodium chloride. The concentration of gamma globulin in this solution was established by repeated micro-Kjeldahl analyses; volumetric dilutions of this standard were then prepared. Quantitative precipitin determinations were carried out by adding 1 ml. of each antigen dilution to 1 ml. of antiserum in standard heavy-walled precipitin tubes. After thorough mixing by agitation, the tubes were capped with parafilm, and allowed to incubate for one hour at 37° C., then placed at 0 to 4° C. for eight days. The tubes were agitated daily. They were then centrifuged for one hour in the cold at 3,000 rpm, and the supernates poured off and tested for the presence of antigen and antibody. All procedures were carried out in the cold. The tubes were allowed to drain over filter paper for 15 minutes, and the precipitates were washed by resuspension in 0.145 N nitrogen-free NaCl. This washing procedure was repeated twice. The washed specific precipitates were digested with 0.5 ml. digestion solution and analyzed for nitrogen by the micro-Kjeldahl method. Repeated analyses, using the same as well as different lots of antiserum, yielded results agreeing within less than 5 per cent in all instances, and in most instances, the agreement was within less than 2 per cent. Reference was thenceforth made to the zone of antibody excess in the precipitin curves thus constructed. One hundred-fifty ml. of rab-

TABLE I

Serum gamma globulin concentrations in normal humans: Immunochemical estimations

Group	Number	Gamma globulin	Range
		mg. %	
Normal infants 1 day–7 wks.	13	663.19	417.90–1140.62
Normal infants 3–6 mo.	9	398.3	214.84– 621.09
Normal infants 6–15 mo.	14	731.2	279.69–1125.00
Normal children 19–54 mo.	18	882.37	440.62–1541.60
Normal children 7–15 yrs.	6	1151.9	833.40–1312.50
Normal adults 19–34 yrs.	24	1124.3	734.00–1687.50

bit anti-human gamma globulin serum was treated with 75 mg. of lyophilized agammaglobulinemic serum which had been found to contain 2.5 mg. per cent of gamma globulin, incubated for one hour at 37° C. and then stored at 0 to 4° C. for eight days, with daily agitation. Sterile procedure was used throughout. The treated antiserum was then centrifuged at 3,000 rpm for one hour in the cold and decanted. Seventy-five ml. of this once absorbed antiserum was again absorbed by the same technique. Finally, aliquots of the unabsorbed, once and twice absorbed antisera were decomplemented with crystalline BSA-rabbit-anti-BSA.[1] The latter reagents were added in precise proportions corresponding to their equivalence point as determined by previous quantitative precipitin studies. Separate quantitative precipitin determinations were then performed using each of the antisera with dilutions of the standard gamma globulin solution.

Figure 1 demonstrates the close agreement among the amounts of gamma globulin precipitated in the zone of antibody excess with the three antisera, and serves to demonstrate that even the unabsorbed antiserum possessed sufficient immunological homogeneity for use in determinations of gamma globulin concentrations. As may be seen from the figure, no decrease in the amount of precipitate occurring in the zone of antibody excess took place when absorbed serum was used. These findings also suggest that the gamma globulin preparation used as antigen did not contain other protein constituents in significant amounts.

Free electrophoresis was carried out in a Klett Model E Tiselius apparatus at 4° C. against veronal buffer, pH 8.6, ionic strength 0.1; the gamma globulin used as antigen was found to be symmetrically monophoretic. Paper electrophoresis was carried out in a Spinco Model R series B paper electrophoresis apparatus, and the patterns obtained in the Spinco Analytrol. This study, too, revealed that the gamma globulin fraction employed as antigen was homogeneous.

[1] Armour and Co., bovine plasma albumin, crystallized.

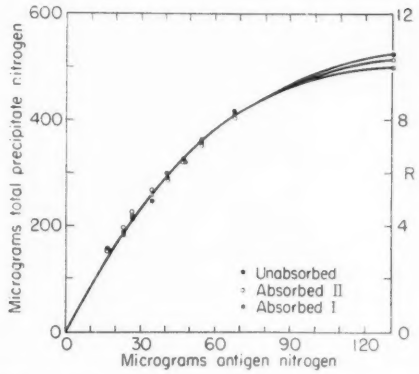

FIG. 1. ABSORPTION-PRECIPITIN STUDY OF RABBIT ANTI-HUMAN GAMMA GLOBULIN SYSTEM

Studies performed with Oudin (18), double diffusion, and Ouchterlony (18) methods revealed a single band of precipitate at equivalence when the antiserum was tested against the standard human gamma globulin antigen, normal human sera and agammaglobulinemic sera.

RESULTS AND DISCUSSION

Summarized in Table I are the gamma globulin concentrations as determined immunochemically on normal infants, children and adults. The distribution of gamma globulin levels is also shown as the stippled area on Figure 3.

Table II describes the quantitative immunological deficit in 16 agammaglobulinemic patients. All agammaglobulinemic patients have some gamma globulin as measured in this way; the serum gamma globulin concentrations in patients with congenital agammaglobulinemia ranged from 2.5 to 16.0 mg. per cent while those of patients with acquired agammaglobulinemia ranged from 12.0 to 97.0 mg. per cent. Although some overlap between the two groups was observed, it will be seen that the adult agammaglobulinemics generally possess higher concentrations of gamma globulin in their serum than do the children with the congenital form of the disease.

Figure 2 summarizes the data obtained in the immunochemical estimation of the biologic half-life of parenterally administered gamma globulin in patients with agammaglobulinemia. In this study, serum gamma globulin concentrations were plotted as a semilogarithmic function of time following the injection of various doses of gamma globulin. These patients ranged in age from one

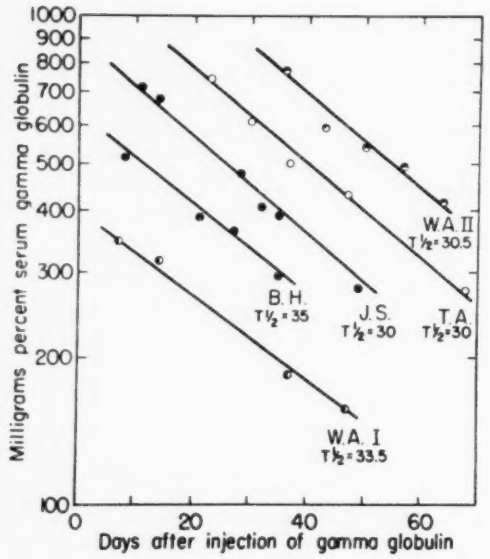

FIG. 2. BIOLOGIC HALF-LIFE OF HUMAN GAMMA GLOBULIN IN PATIENTS WITH AGAMMAGLOBULINEMIA

to seven years. The biologic half-life of gamma globulin in these patients ranged from 30 to 35 days and appeared to be independent of age, concentration of the protein, or time after injection.

Figure 3 describes the appearance of gamma globulin in the normal female offspring of a patient with acquired agammaglobulinemia, and its decline in the agammaglobulinemic male offspring of an apparently normal female. In the first instance, the gamma globulin concentration of the maternal blood did not differ significantly from that of the cord blood, nor were the isohemagglutinins or antibodies to any of the antigens with which the mother had been stimulated observed in the baby's serum. Antigenic stimulation was provided the baby by weekly injections of typhoid-paratyphoid vaccine, and diptheria, pertussis and tetanus vaccine during the first two months and monthly thereafter. The baby was born agammaglobulinemic and remained so throughout the first 40 days of life. On approximately the fortieth day of life, the serum gamma globulin concentration was found to be 115 mg. per cent and it rose rapidly thereafter. Eighteen days following the first increase in gamma globulin concentration and at a time when the concentration of this

TABLE II

Immunochemical estimations of serum gamma globulin concentrations in patients with agammaglobulinemia

Form of disease, Subject	Sex	Age	Gamma globulin concentration
		yrs.	*mg. %*
Congenital agammaglobulinemia			
T. A.	M	1	14.0
J. S.	M	2	2.5
W. A.	M	6	4.4
B. H.	M	6	10.0
E. S.	M	7	11.5
R. J.	M	3	16.5
R. D.	M	3	13.4
S. E.	M	11½	8.8
J. Si.	M	1	10.0
			Mean: 10.1
			Range: 2.5–16.0
Acquired agammaglobulinemia			
L. L.	F	30	12.0
F. H.	M	58	40.0
K. N.	M	35	97.0
A. L.	M	20	65.5
L. Z.	F	56	30.0
M. O'B.	F	63	19.0
B. A.	F	67	74.0
			Mean: 48.2
			Range: 12.0–97.0

protein was nearly 200 mg. per cent, antibodies to H antigen were first demonstrated in significant titer. Thereafter, the baby formed agglutinins to 0 and B antigens, formed diphtheria antitoxin, and became Schick negative at four months of age. It is of interest that appreciable quantities of gamma globulin were present for a considerable period preceding the appearance of demonstrable levels of circulating antibodies.[2]

The decline of maternally transmitted gamma globulin in the agammaglobulinemic son of a normal female emphasizes several points of interest. First, this child who cannot form gamma globulins begins life with at least a normal concentration of this protein in the serum. The logarithmic decline of gamma globulin in this baby was uniformly linear throughout the first 10 months of

[2] Since this study has been completed, another female child was born to the same agammaglobulinemic mother. Similar studies of gamma globulin concentration revealed a curve almost identical to that of the baby described here except that the initial increment in circulating gamma globulin concentration occurred between 20 and 28 days after birth rather than between 35 and 42 days as was the case with this baby.

life, at which time the serum concentrations fell within the range observed among other agammaglobulinemic children. The half-life of gamma globulin in this child throughout the first 10 months of life is comparable to that seen in agammaglobulinemic patients given gamma globulin parenterally.

In Figure 4 are shown semilogarithmic plots of the decline of gamma globulin concentration of a normal offspring of a patient who had previously borne a son found to be agammaglobulinemic. In this graph, the decline of gamma globulin concentration is compared to the decline observed following injection of gamma globulin in a six year old agammaglobulinemic boy. During the first 80 days of life, the curve in this patient was similar in slope to that defining the half-life of injected gamma globulin in the agammaglobulinemic patients and virtually identical to that defining the decline in gamma globulin concentration in an agammaglobulinemic baby born of an apparently normal mother (Figure 3).

Table III summarizes the serum values on 31 maternal blood-cord blood pairs taken at parturition. The differences between the two groups of sera with respect to total protein concentration and gamma globulin concentration estimated both electrophoretically and immunologically were small. Although the gamma globulin concentrations determined by the immunochemical method were slightly higher in the cord serum than in the maternal serum, the difference between the two groups was not statistically significant. These observations, although consistent with their interpretations, provide little support for the conclusions of Moore, Martin du Pan and Buxton, and others who suggest that active transport and/or local placental production of gamma globulins are responsible for elevated cord levels noted with electrophoretic methods (19).

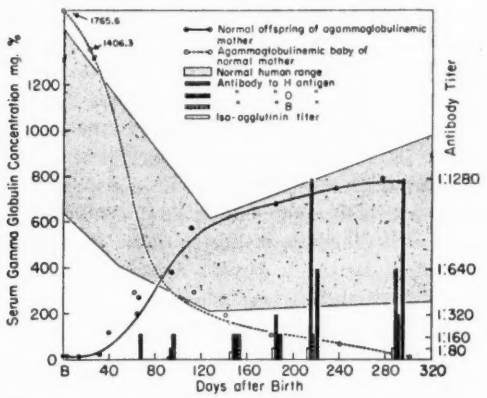

FIG. 3. APPEARANCE OF GAMMA GLOBULIN AND ANTIBODY SYNTHESIS IN THE NEWBORN PERIOD

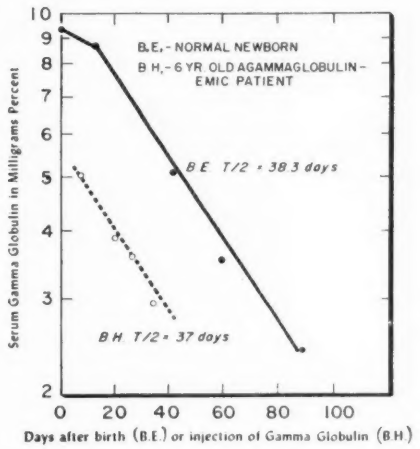

FIG. 4. BIOLOGIC SURVIVAL TIMES OF GAMMA GLOBULIN IN A CONGENITAL AGAMMAGLOBULINEMIC PATIENT COMPARED WITH DECLINE IN A NORMAL NEONATE

DISCUSSION

The immunoelectrophoretic observations of Grabar and Williams (20, 21) indicate that human gamma globulins may be immunologically homogeneous and physico-chemically heterogeneous. The immunological studies in the agar precipitin systems performed here support this contention. Furthermore, the absence of significant amounts of serum proteins other than gamma globulins from the material used as the antigen in these studies, as well as the specificity of the antibodies produced in the rabbit against this human gamma globulin, is suggested by the absorption-precipitin study.

Previous studies comparing results of the gamma globulin levels obtained with the immunochemical method and gamma globulin levels obtained by electrophoretic analyses have revealed that higher concentrations are obtained with the former technique. This finding has been variously attributed to the presence of gamma-reactive globulin under

TABLE III

Serum values on 31 maternal-cord samples taken at delivery

	Total serum protein		Electrophoretic gamma globulin		Immunochemical gamma globulin	
	Maternal	Cord	Maternal	Cord	Maternal	Cord
	Gm. %		*mg. %*		*mg. %*	
Mean	6.965	6.529	940.4	1139.7	978.7	1081.8
S. D.	0.81	1.28	264	320.8	296.4	285.9
Range	5.6–9.3	4.85–10.2	580–1826	510–2070	509.4–1762.5	645.3–2187.5
p Value	0.1096		0.0076		0.1645	

the alpha and beta globulin peaks of the electrophoretic spectrum. When paper electrophoretic methods are used, albumin tailing, higher affinity of the gamma globulins for the dyes used, and the application artifact introduce errors which prohibit strict quantitation. The data presented here, however, in agreement with those of Goodman and co-workers (*22*), suggest that immunochemical and electrophoretic values are of the same order of magnitude within the usual ranges of concentrations observed in human sera. The immunochemical method, being more sensitive and more precise, affords clearer insight into subtle differences in protein concentrations, and is particularly valuable in assessing very low concentrations of protein.

Among the sera of patients suffering from the severe handicap in protein synthesis, agammaglobulinemia, we have observed no instance in which gamma globulin was not detectable by precipitin methods in liquid-liquid or in semisolid media. The finding of extreme hypogammaglobulinemia rather than complete absence of the protein in the sera of these patients by immunochemical means was reported upon in the original description of the disease. Our observations support this conclusion and indicate that neither impurities in antigens used nor cross reactivity among the serum proteins account for the small amounts of gamma globulin measured in each case.

The mean biologic half-life of parenterally administered gamma globulin, established by the immunochemical method, reveals a value which is comparable to that reported by Bruton, Apt, Gitlin and Janeway (*11*) in agammaglobulinemics by a similar technique, and by others in normal humans using immunologic, electrophoretic and salt precipitation techniques, respectively (*23–25*).

The appearance in the newborn of apparently nonimmune, gamma-reactive globulin in appreciable quantities well before the appearance of specific serum antibody might suggest that only some gamma globulin is antibody. However, the existence of this apparently nonimmune globulin may well represent a response to antigens which are ubiquitous and for which testing was not accomplished. The failure of this child to synthesize gamma-reactive globulin during the first 40 days of life is also apparent from these data. Further data collected in this laboratory on a similar case revealed initial appearance of serum gamma globulin between 21 and 28 days of life.

The immunochemical studies reported herein are in essential agreement with other studies performed using different methods to reveal the variation in serum gamma globulin concentrations throughout infancy and childhood. Normal infants display a variable interval, lasting between two and six months, prior to the assumption of full immunological responsibility. During this interval, the gamma globulin concentrations may decline normally to values slightly above 200 mg. per cent. Certainly most children show no apparent ill effects of the immunological inadequacy or the low gamma globulin levels. Failure to assume active gamma globulin synthesis, or delay in development of this function, is followed by further decline in gamma globulin concentration. This state has been referred to as the transient hypogammaglobulinemia of infancy and is characterized clinically, just as is clinical agammaglobulinemia, by extreme susceptibility to bacterial infection. Similarly, a child subsequently proved to be agammaglobulinemic showed con-

tinued decline in gamma globulin concentration at an almost identical rate to that observed early in the neonatal period in children subsequently shown to be immunologically normal. In this instance, however, the decline continued until levels under 10 mg. per cent were reached. This child developed increased susceptibility to infection when the gamma globulin level descended below 100 mg. per cent (Figure 3). From these and other observations, it would appear that the critical level of serum gamma globulin concentration is of the order of 100 mg. per cent.

SUMMARY

Procedures for the quantitative estimation of gamma globulins in human sera used in this laboratory are presented. Data suggesting the adequate homogeneity of a gamma globulin preparation for use in immunochemical estimations are presented. Normal values for the gamma globulin concentration in newborns, infants, children and adults, as determined by immunochemical methods, are presented and compared with electrophoretic values. Gamma globulin concentrations in 16 patients with agammaglobulinemia are shown. The half-life of injected gamma globulin in four congenitally agammaglobulinemic patients, as determined by the immunochemical method, is documented. The assumption of gamma globulin synthesis and immunological responsibility in the unique instance of a normal offspring of an agammaglobulinemic patient is described and compared with a "reciprocal" instance of an agammaglobulinemic offspring of an immunologically normal female. The rate of decline of gamma globulin concentration during the first two and one-half months of life observed in an immunologically normal child was found to be similar to that of parenterally injected gamma globulin in agammaglobulinemic patients and similar to the decline of gamma globulin concentration observed during the neonatal period in an agammaglobulinemic child.

ACKNOWLEDGMENTS

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THE EFFECTS OF CHEST IRRADIATION ON PULMONARY FUNCTION * †

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Pathological changes in lung tissue following chest wall irradiation have been adequately documented in the past (1–5). However, pulmonary function has not been systematically studied. Published studies (6–9) are incomplete and the findings complicated by the original disease. In view of this and because of a desire to determine the amount of radiation which might safely be administered to the chest, a systematic study was undertaken in dogs. The results of this study follow.

METHODS

Pulmonary function and vascular resistance were evaluated in dogs before and after irradiation to the chest. All studies were performed under pentobarbital anesthesia. Two different irradiation schedules were utilized. Eight dogs received a calculated dose of 1,000 to 2,900 roentgens (r) to the mid-chest in a single exposure. No animal survived longer than two and one-half months. For practical reasons, it was not feasible to study resistance and function in the same animal. Therefore, resistance was evaluated in the first five animals within 24 hours of irradiation. Diffusing capacity, functional residual volume and compliance were determined in the remaining three animals at two to three week intervals. In order to effect a longer period of survival, seven animals were irradiated under a second schedule consisting of a dosage of 200 to 300 r to each side of the chest repeated at weekly intervals up to a total calculated mid-chest dose of 3,000 to 4,800 r. Three of these animals survived longer than six months. Diffusing capacity, functional residual volume and compliance were determined at two to three month intervals following onset of irradiation. Vascular studies were also conducted in four animals at the time of the final evaluation.

All irradiation was given with 260 KV. peak equipment (filter, one-half mm. Cu, 1 mm. Al, half-value layer 1 mm. Cu; target-skin distance, 50 to 70 cm.) with the animals under pentobarbital anesthesia. Field size included the chest from xiphoid to lower neck.

Compliance. Values for total thorax, lung and chest wall compliance were obtained by the static method (10) with the animals anesthetized and curarized. A cardiac catheter, with a latex condom secured to the terminal 5 inches, was introduced well into the thoracic portion of the esophagus (11, 12). The catheter was marked at the level opposite the incisors, so that it could be placed in the same position in subsequent tests. A cuffed endotracheal tube was positioned within the trachea and sealed by inflation of the cuff. Muscle paralysis was obtained by the intravenous injection of approximately 6 ml. of a 0.01 per cent solution of succinyl-choline. Respiration was maintained artificially with a mechanical respirator, except during evaluation of the pressure-volume relationships. Air was introduced into the tracheal cannula until pressure in the cannula reached a pre-selected level. In order to minimize variations due to hysteresis, inflation time for a given volume was maintained approximately equal at each study. Tracheal and esophageal pressures were recorded. The lungs were then allowed to deflate passively into a recording spirometer. After 15 to 20 seconds the procedure was repeated. Tracheal pressures of 10, 20, 30, 40 and 50 cm. of H_2O were utilized. Three determinations were made at each pressure. Compliance was calculated as follows:

$$\text{Total thorax compliance} = \frac{\text{Volume (L. ATPS)}}{\text{Tracheal pressure (cm. } H_2O)}$$

$$\text{Thoracic cage compliance} = \frac{\text{Volume (L. ATPS)}}{\text{Esophageal pressure (cm. } H_2O)}$$

$$\text{Lung compliance} = \frac{\text{Volume (L. ATPS)}}{\text{Tracheal-esophageal pressure (cm. } H_2O)}.$$

In order to determine the accuracy of values for lung compliance calculated from the above formula, the chest walls of three animals were opened and retracted, and compliance of the lungs was determined directly. Values obtained with the chest open were compared with those calculated from data obtained with the chest closed.

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‡ Clinical Investigator of the Veterans Administration.

Functional residual capacity (FRC). Functional residual capacity was determined by the open circuit method during spontaneous breathing (13, 14). Respiration was monitored continuously during the procedure with a nitrogen-analyzer [1] to detect completeness of washout. Washout of nitrogen was complete in three minutes. Gas collection was continued for two minutes longer. The sample volume was measured in a spirometer and the per cent of nitrogen estimated by the analyzer. FRC was calculated according to the usual formula (14).

Diffusing capacity. Diffusing capacity was estimated by the steady state carbon monoxide method during spontaneous breathing (15). Equilibration and sample collection times were two minutes each instead of the three minutes suggested in the reference article. Arterial blood oxygen and carbon dioxide tensions were determined by the bubble equilibrium technique (16). Expired gas samples were analyzed for carbon monoxide and carbon dioxide with infrared analyzers.[2] The oxygen percentage was determined with an oxygen analyzer.[3] Total gas volume, including portions analyzed, was spirometrically measured and converted to STPS. Minute ventilation was obtained by dividing the total gas volume by collection time. Diffusing capacity (Dco) was calculated from the original equations (15). Duplicate determinations were made in three animals in order to determine the reproducibility of the method. The second determination was 78, 80 and 103 per cent of the first in the three animals, respectively. Single determinations were made in all subsequent examinations.

Pulmonary vascular studies. Cardiac catheters were inserted into the pulmonary artery and left atrium via the jugular vein and carotid artery, respectively. These techniques have been described elsewhere (17). Pressures were measured with resistance wire pressure transducers. Cardiac output was calculated by the Fick principle. The per cent of oxygen in expired air was measured with the oxygen analyzer. Oxygen consumption was obtained from the product of the percentage difference in oxygen between inspired and expired air and the corrected total one minute expired volume. Pulmonary vascular resistance was calculated by dividing the difference in pressure between the pulmonary artery and left atrium by the cardiac output.

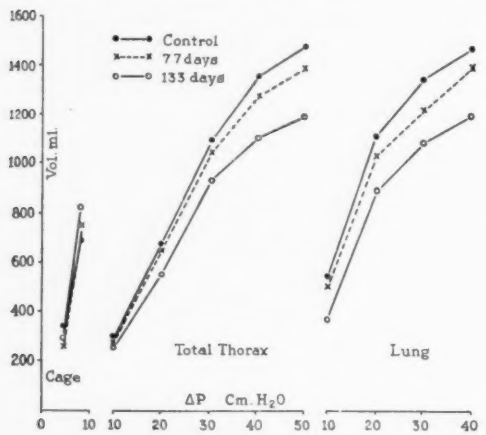

FIG. 1. AVERAGE RELATION BETWEEN PRESSURE AND VOLUME IN CAGE, LUNGS AND TOTAL THORAX BEFORE AND AFTER FRACTIONAL IRRADIATION TO THE CHEST IN SIX ANESTHETIZED CURARIZED DOGS

Three volume measurements were made at each pressure in each animal.

[1] Model A-5, Waters Conley Company, Rochester, Minn.

[2] Liston-Becker Model 16, CO_2 Analyzer, Beckman Instruments, Inc., Belmont, Cal. Model 15A, CO Analyzer, Beckman Instruments, Inc., Springdale, Conn.

[3] Model E-2, Arnold O. Beckman, Inc., 1020 Mission Street, South Pasadena, Cal.

RESULTS

Compliance

The values for total thorax compliance increased progressively as the pressure was elevated to 30 cm. H_2O (Figure 1). Further pressure elevation resulted in a reduction in compliance. The same relationship was apparent for the lung. These findings are similar to those reported by others (10, 18).

Total compliance decreased following irradiation mainly as a result of a reduction in lung compliance. Three animals were studied two and four weeks following single dose irradiation. Changes were irregular at two weeks. By four weeks, however, reduction in both total and lung compliance was apparent in two of the three animals. Six dogs were studied an average of 77 and 133 days following onset of fractional irradiation (Figure 1). Control values for total thorax compliance at 20 cm. H_2O averaged 0.034 L. per cm. H_2O. By 77 days, total compliance had decreased in three of the six animals and the average value was 0.033. By 133 days, it had decreased in all six and the average value was 0.028 ($t = 2.92$, $p = 0.05$ to 0.01). The changes in lung compliance were similar. The average control value at 20 cm. H_2O was 0.056. By 77 days, lung compliance had decreased in four of the six animals and the average value was 0.052. By 133 days, it had decreased in all six and the average value was 0.045 ($t = 3.36$, $p = 0.05$ to 0.01). Cage compliance did not change greatly

in any animal. These findings indicate that the increased rigidity of the lungs-chest resulted from changes within the lung. Three of these animals were restudied 172, 228 and 273 days, respectively, following onset of irradiation. Total and lung compliance was further decreased in all three. Thoracic cage compliance was below the pre-irradiation value in only one of the three (Figure 2).

One nonirradiated and two irradiated dogs were studied immediately before and after opening the chest. The values obtained with the chest open compared favorably with those with the chest closed (Figure 2).

Diffusing capacity

Diffusing capacity did not change consistently in three animals studied two weeks and four weeks following irradiation with a single dose. It decreased progressively in those animals studied more than two months following onset of fractional irradiation (Table I, Figure 3). Control values from seven animals averaged 4.3 ml. per minute per mm. Hg. By 82 days following the onset of irradiation, diffusing capacity was below the control value in five, above it in one and the same in one. The average value was 3.2 ml.

TABLE I

Values for several measures of pulmonary function before and at various time intervals after the onset of fractional irradiation to the chest

Dog no.	Weight and sex	Time of study	FRC	Min. vent.	pCO_2	pO_2	Dco
		days	*ml.*	*L./min. STPD*	*mm. Hg*	*mm. Hg*	*ml. CO min./ mm. Hg*
1	38.5 lbs. Male	C*	650	2.5	45.6	74.5	5.0
		80	609	4.2	51.9	71.1	2.3
		126	490	1.8	20.5	65.2	1.5
2	49 lbs. Male	C	678	4.7	48.7	82.9	3.7
		104	615	1.9	54.6	68.5	3.4
		161	492	2.9	48.1	47.1	1.5
7	45 lbs. Male	C	754	2.4	45.6	90.0	3.7
		97	269	2.0	38.3	84.2	3.7
		147	332	3.8	28.9	69.9	2.0
8	32 lbs. Male	C		3.4	31.6	101.1	3.5
		74		3.4	35.9	91.9	3.6
		141	465	2.2	44.4	95.0	2.8
12	48 lbs. Male	C		2.1	43.5	77.7	4.4
		87	566	3.9	30.3	86.2	3.1
		138	546	2.8	33.1	99.5	2.4
		273	344	3.9	33.5	81.7	3.6
13	46.5 lbs. Male	C		7.2	38.3	84.4	5.7
		64	545	2.3	41.6	77.7	3.1
		104	509	1.9	47.9	80.7	2.0
		228	388	9.7	50.1	33.9	1.0
14	41 lbs. Female	C	519	4.3	33.0	81.1	3.9
		70		2.3	51.3	92.4	3.0
		140	513	2.9	34.6	89.6	2.0
		172	418	3.1	53.0	80.6	4.1
Average		C		3.8	40.9	84.5	4.3
		82		2.9	43.4	78.9	3.2
		137		2.6	36.8	78.1	2.0

* C, control.

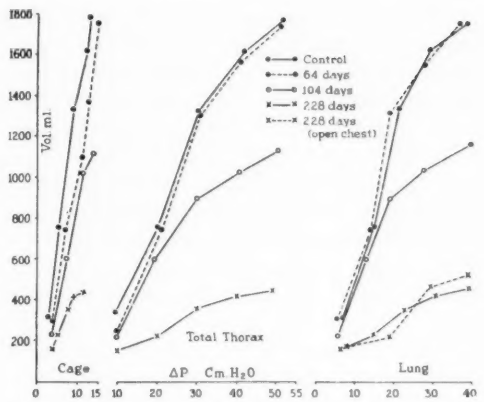

FIG. 2. RELATION BETWEEN PRESSURE AND VOLUME IN CAGE, LUNGS AND TOTAL THORAX OF DOG NO. 13 BEFORE AND AT THREE PERIODS AFTER FRACTIONAL IRRADIATION TO THE CHEST

On the two hundred twenty-eighth day, values for the lung were also obtained immediately after opening the chest. Each point represents the average of three measurements.

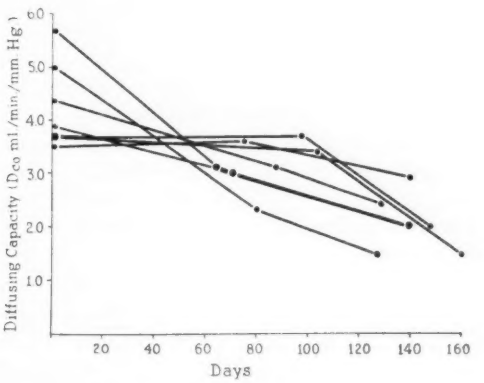

FIG. 3. CHANGE OF PULMONARY DIFFUSING CAPACITY IN SEVEN DOGS FOLLOWING FRACTIONAL IRRADIATION TO THE CHEST

Each point represents one determination.

per minute per mm. Hg ($t = 2.56$, $p = 0.05$ to 0.01). By 137 days, diffusing capacity was below the control value in all seven. The average value was 2.0 ml. per minute per mm. Hg ($t = 5.68$, $p = < 0.01$). Three of these animals were studied 172, 228 and 273 days, respectively, following onset of fractional irradiation. Diffusing capacity further decreased from the preceding value in one, but increased toward normal in two.

A low diffusing capacity was frequently associated with a low pO_2, and in occasional instances this occurred despite a decrease in pCO_2 and minute ventilation. The changes in minute ventilation, pCO_2 and pO_2 were not regular, however, and are only included for the sake of completeness (Table I).

Functional residual capacity

There was no change in the functional residual capacity in three animals studied two and four weeks following irradiation with a single dose. Functional residual capacity decreased in all animals studied more than 80 days following onset of fractional irradiation (Table I). Pre-irradiation values from four dogs averaged 650 ml. This value, on a weight basis, is similar to that reported by Simmons and Hemingway (14). Eighty-one and 137 days following irradiation, average values were 521 and 478 ml. Functional residual capacity further decreased in three animals restudied more than 172 days following onset of fractional irradiation.

Pulmonary vascular resistance

Table II shows that pulmonary arterial pressure, pulmonary venous pressure, cardiac output and pulmonary vascular resistance did not change significantly up to a period of five months following irradiation. In three animals studied after six months, pulmonary arterial pressures and resistances appeared to be elevated.

Values for resistance before and at three and 24 hours after irradiation averaged 2.1, 3.0 and 2.0 mm. Hg per L. per minute, respectively, in five animals. Re-evaluation of one animal 80 days after irradiation revealed a value of 3.2 mm. Hg per L. per minute. Two additional dogs were studied 68 and 135 days after single and fractional dose irradiation, respectively. Resistance values in these two animals were 0.8 and 1.0 mm. Hg per L. per minute. Three dogs were studied more than six months following onset of fractional irradiation. Comparison of these values (Table II) with the control values from other animals in this study and with those reported elsewhere (19) indicate that two of these values were questionably elevated and one was definitely elevated. The animal with the highest value for pulmonary vascular resistance (No. 13) also had the greatest changes in compliance and diffusing capacity as well as the most marked histological changes.

TABLE II

Pulmonary vascular variables immediately before and at various time intervals following onset of irradiation to chest

Dog no.	Time of study	Art. O_2	Ven. O_2	Cardiac output	Mean pul. art. pres.	Mean pul. ven. pres.	Pul. vas. resist.
	days	*vol. %*	*vol. %*	*L./min.*	*mm. Hg*	*mm. Hg*	*mm. Hg/ L./min.*
1A	C*	11.0	16.0	3.4	28.0	24.0	1.2
	0.1†	13.1	16.3	6.5	19.0	6.0	2.0
2A	C	14.5	17.7	5.7	17.5	10.8	1.2
	0.1‡	13.3	16.9	4.7	13.7	9.3	0.9
3A	C	18.4	20.6	5.1	16.5	3.5	2.6
	0.1‡	20.3	24.7	2.7	20.0	3.0	6.3
	0.9	17.3	20.0	6.2	15.5	2.0	2.2
5A	C	13.2	15.9	4.3	12.5	3.5	2.1
	0.1‡	12.5	15.9	3.5	14.5	2.5	3.5
	0.9	9.0	12.0	4.0	10.0	4.0	1.5
	80.0	13.8	18.2	1.8	7.0	1.5	3.2
6A	C	17.1	20.4	2.6	12.5	4.0	3.3
	0.1‡	16.6	20.3	2.4	8.5	3.5	2.1
	0.9	16.8	20.3	3.0	12.5	5.5	2.4
11	68.0§	14.7	17.7	5.8	9.0	4.5	0.8
8	135.0‖	10.5	17.6	2.5	11.0	4.5	1.0
12	273.0¶	14.1	18.7	2.8	22.0	4.5	6.2
13	228.0¶	12.1	18.5	0.8	30.8	1.9	38.0
14	172.0¶	13.4	19.5	1.4	18.2	1.4	11.6

* Control.
† Time after 2,000 r single dose.
‡ Time after 3,000 r single dose.
§ Time after 2,900 r single dose.
‖ Time after starting 4,800 r fractional dose.
¶ Time after starting 3,000 r fractional dose.

Pathology

The lungs of 11 animals were examined microscopically. The findings four days after a single

dose of irradiation revealed little abnormality except for capillary dilatation. No changes were seen in the larger vessels. Four to five months after fractional irradiation, the histological findings demonstrated focal atelectasis with some fibrosis and hyperemia of the interstitial areas. Chest roentgenograms taken regularly during the study period failed to reveal any obvious abnormalities.

In the three animals studied more than 172 days following the onset of irradiation, obvious, interstitial fibrosis with paucity of cellular elements and capillaries was apparent. The small pulmonary arteries of Dog No. 13 revealed marked narrowing due to endothelial proliferation. There was also focal necrosis of the walls, and few areas of acute hemorrhage. The lymph vessels were noticeably dilated.

DISCUSSION

The present study demonstrates that irradiation of the chest of the dog with the dosage employed is followed by progressive reduction in pulmonary diffusing capacity, lung compliance and functional residual volume.

The study failed to demonstrate significant changes in any of the physiological variables measured during the first few weeks following irradiation. Pathological studies indicate that hyperemia, edema and thick secretions occur with some regularity during the early period following irradiation (2). This discrepancy between pathological and physiological findings might result simply because measurements were made at inappropriate times or because the physiological changes were too small to be detected by the methods utilized. On the other hand, the discrepancy may also be explained on physiological grounds. For example, active constriction might produce changes in various resistance and diffusion components that would not be detected by measurements of total resistance and total diffusion. Pulmonary venular constriction is known to occur following inhalation of steam (20) or injection of endotoxin (21). As a result of increased resistance to flow through venules, hydrostatic pressures increase proximally. These elevated pressures passively distend capillaries and other small vessels, thereby reducing their resistance to flow. Caliber changes which are directionally opposite might therefore account for the absence of a significant change in total resistance. Venular constriction also increases capillary blood volume and promotes edema formation (22). Edema may result from capillary hydrostatic pressures in excess of colloid osmotic pressures, increased capillary surface area or increased permeability due to the noxious effect of irradiation on capillary walls. A normal total diffusing capacity might be expected since interstitial edema and increased blood volume have opposite and cancelling effects upon total diffusing capacity (23, 24). A differential diffusion method such as the one decribed by Forster and associates (25, 26) might detect such changes. On the other hand, the absence of measurable compliance and functional residual capacity changes during this early period argues against venular constriction of a severe degree. Elevated blood volume and edema would be expected to decrease compliance (27) and functional residual capacity.

All measured variables appeared to change significantly within six months following onset of fractional irradiation. Compliance, diffusing capacity and functional residual capacity probably decreased by 80 days and definitely decreased by 140 days. Pulmonary vascular resistance seemed to increase at about six months. These physiological changes apparently result from the interstitial fibrosis, cellular infiltration and reduced cross-sectional area of the pulmonary vessels which were observed at autopsy. Arguments for this statement follow: It is possible that focal atelectasis may in part account for the reductions in compliance and diffusion. That it is not the entire explanation is indicated by the fact that these functions were reduced in some anmials in which significant atelectasis was not observed. Variation in minute ventilation, due to different depths of anesthesia, was at first considered as a possible cause of the reduction in diffusing capacity. It soon became apparent, however, that there was little correlation between the two parameters (Table I). It is unlikely that the observed changes in diffusing capacity are related to a failure to correct for COHb in the blood. Such a correction would significantly influence absolute values but could hardly account for changes with time in a given animal. The changes in diffusing

capacity probably were not the result of changes in the level of hemoglobin. The hemoglobin levels (mean ± S. D.) before irradiation and at 2, 4 and 7 month intervals after irradiation were 14.8 ± 0.9, 12.9 ± 1.3, 12.5 ± 1.2 and 13.8 ± 1.3 Gm. per 100 ml., respectively. Hence, the change in pulmonary function was probably the result of the pathological changes observed at autopsy.

The values for diffusing capacity reported in this paper are considerably lower than those reported for four dogs by Otis and Jude (28). However, their methods differed from those in the present study. Otis and Jude maintained respiration artificially during their measurements and used a different technique to sample expired volume. These differences might tend to elevate diffusing capacity. The present study offers some evidence to support this statement. Diffusing capacity was determined in two animals, each ventilated with tidal volumes of 250 and 500 ml. Diffusing capacity with the lower volume was 4.3 and 4.0 ml. per minute per mm. Hg, respectively. With the higher volume the corresponding values were 14.0 and 10.3 ml. per minute per mm. Hg. respectively. These differences might be related to changes in diffusing area and vascular volume due to differences in airway pressures.

Total compliance decreased almost entirely because of decrease in lung compliance. Chest wall compliance decreased measurably in only one animal and its contribution to the change in total compliance was minimal. Increased rigidity of the lung may result from cellular infiltrate, fibrosis and atelectasis. All were observed in the present study and have also been reported by others. Fibrosis, cellular infiltrate, edema, atelectasis and reduction of pulmonary capillary blood volume all probably contributed to the progressive reduction in diffusing capacity. The latter most likely accounts for the tendency to a progressive decrease in arterial oxygen tension. The return of diffusion capacity toward normal in two animals may be related to clearing of cellular infiltrate or edema fluid. The most likely explanation of the progressive decrease in functional residual capacity is fibrous contracture of lungs and chest wall. However, since fibrous contracture might be expected only after the passage of some time and since residual capacity decreased even during the moderately early period following irradiation, other factors might be involved. One such factor might be pulmonary congestion.

An increase in pulmonary vascular resistance could result from passive reduction in vessel caliber by fibrosis and necrosis. Such an increase in resistance to flow would be manifested in an elevated pulmonary arterial pressure for a given rate of blood flow. Freid and Goldberg (7) have noted elevated resistance to flow and right heart failure in humans following irradiation. The present study indicates that at least six months are necessary for these changes to become apparent in the dog. Studies during exercise might have demonstrated vascular fixation earlier. The normal pulmonary vascular bed responds to exercise with a decrease in resistance due, at least in part, to passive distention as pressures increase subsequent to elevation of flow rate. In the presence of fixation, pulmonary arterial pressure would rise to higher levels than normal because resistance would remain constant. The fact that compliance decreased before six months suggests that an increase in fibrous tissue was indeed present even though it was not demonstrated by pulmonary vascular resistance studies made with the animal at rest and anesthetized. This fibrous tissue could decrease the distensibility of the vascular system. Therefore, studies during exercise might have demonstrated fixation before six months. Pulmonary hypertension during exercise in irradiated patients has been reported by Whitfield, Bond and Arnott (29).

SUMMARY

Pulmonary function has been studied in 15 dogs before and at intervals up to nine months following irradiation to the chest. Single doses of 1,000 to 2,900 r and fractional doses of 3,000 to 4,800 r were administered. Pulmonary diffusing capacity, lung compliance and functional residual volume decreased progressively as time elapsed following irradiation. Pulmonary vascular resistance remained normal for a period of five months following irradiation. These physiological changes have been discussed in relation to changes in histology.

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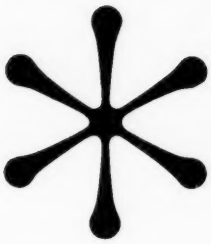

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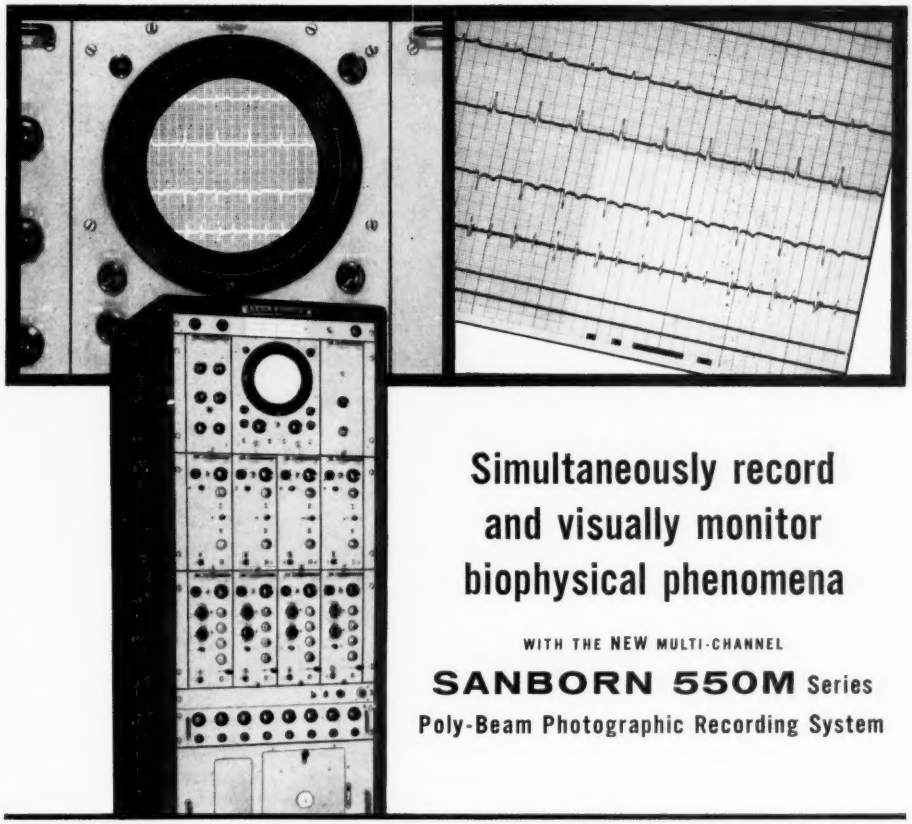

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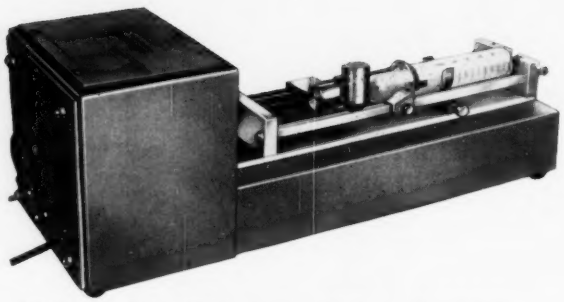

600-900 Infusion/Withdrawal Pump (shown)—equipped with multi-speed transmission which produces 72 separate rates of infusion or withdrawal from 38.4 cc./min. to 0.0008 cc./min. Reproducibility is ± 0.01%. $300.00—f.o.b. Dover, Mass.

900-S Single Speed Infusion/Withdrawal Pump—same as 600-900 but with single speed motor. For dye dilution work, 60 r.p.m. motor is recommended, producing 5 separate rates from 38.4 cc./min. to 4 cc./min. Reproducibility is ± 0.01%. $175.00—f.o.b. Dover, Mass.

Data Sheet 900-Pumps and New Catalog available on request

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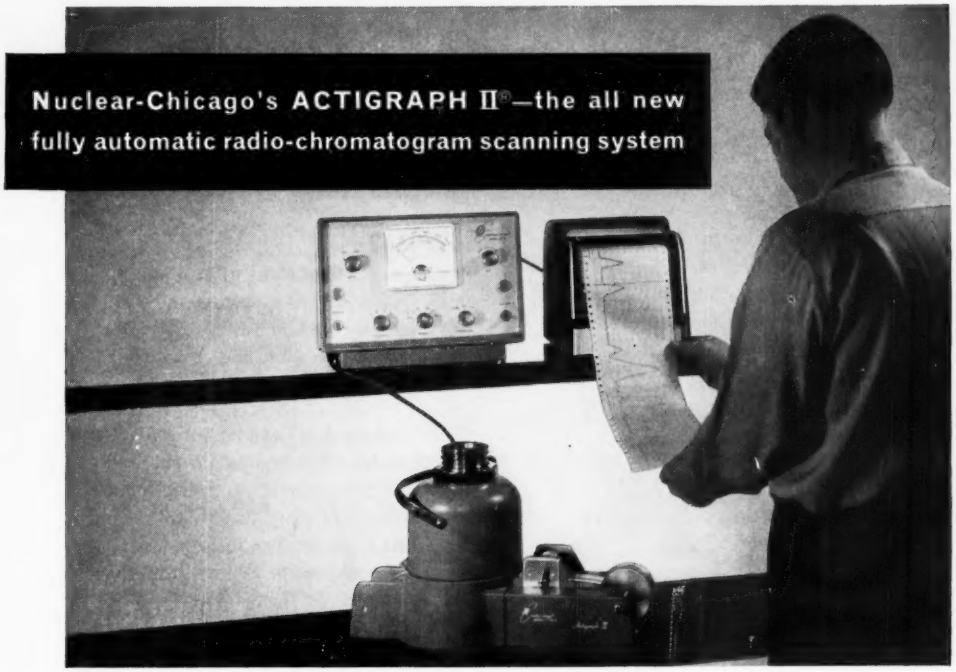
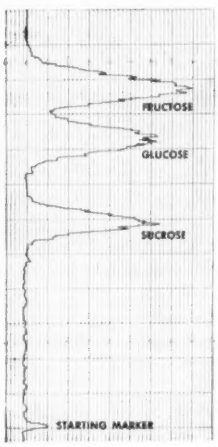
(a non-profit organization)

a new radioisotope counting system for biological and clinical research



Completed chart can be placed next to strip to directly determine location and intensity of radioactive zones.

The new Actigraph II is a completely redesigned and improved scanning system for recording automatically the position and total activity of separated radioactive zones on a strip chromatogram. Two reels accommodate strips up to 50 feet in length allowing single short strips to be spliced end-to-end for continuous operation without attention. A wide choice of detectors may now be used including G-M tubes, *windowless gas flow counters*, and scintillation detectors. Increased sensitivity is provided through improved geometry. The Actigraph II is available with a wide variety of chart recorders including the new "rectilinear" recorder. Chart and strip speeds are easily synchronized by making identical gear changes. For complete information write us and ask for "Nucleus #14".



Typical radio-chromatogram scan made with Actigraph II

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... now more useful than ever in clinical pathology

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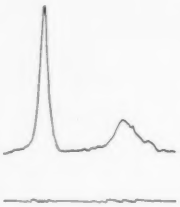
LIPOPROTEINS

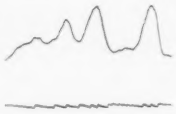


The relationship between these fat-protein molecules and atherosclerosis, liver diseases, diabetes and obesity has made lipoproteins an important field of research.

HEMOGLOBINS

Sickle cell anemia is revealed by the presence on the paper strip of both normal and sickle-cell hemoglobins.

GLYCOPROTEINS



Changes in glycoproteins are often greater and more specific than changes in serum proteins. Indications of glycoprotein correlations are reported in studies of cancer, collagen diseases, acute and chronic infections and tuberculosis.

These new analyses can be performed on the same Model R equipment chosen by most laboratories using paper electrophoresis—equipment which is easy to use and lends itself readily to different analyses, dyes and techniques. For more information, please return the coupon.

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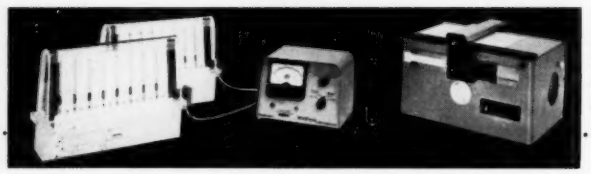

Beckman/Spinco Model R System: cell, power supply and scanner

To: Spinco Division, Beckman Instruments, Inc., Stanford Industrial Park, Palo Alto 16, California

☐ Please send me information on the clinical significance of paper electrophoresis, and a description of the equipment required.

☐ I own a Model R System and would like additional information on recently reported methods.

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MICROPROJECTOR

The extreme brilliance, clarity and definition in screen images from the well-known PRADO make it readily convertible from standard 2"x2" (Model 500) or 2¾"x2¾" (Model 66) slide projector to microprojector.

This portable unit may be carried easily from room to room and used wherever there is an electrical outlet. Light from the 500-watt lamp, projected through aspheric condensers in the blower-cooled PRADO, gives an image quality ideal for use in classroom or conference room.

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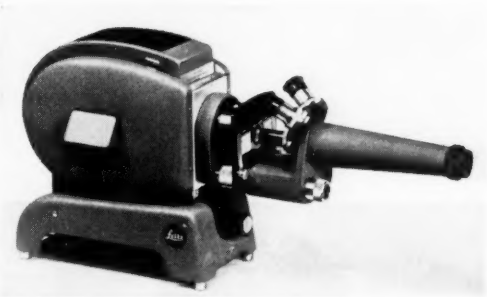
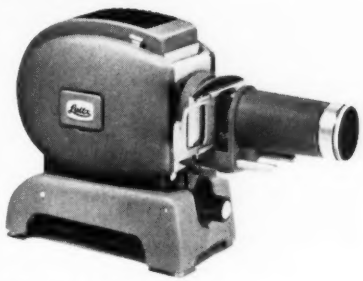


PRADO, as microprojector



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Packard AUTO-GAMMA*

AUTOMATIC GAMMA SAMPLE CHANGER

NOW... Completely Automatic Scintillation Counting!

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Test tubes are 16 mm diameter by 150 mm long for standard well-type scintillation detectors. Any number up to ONE HUNDRED SAMPLES can be counted at each loading. After the last sample in the turntable has been transferred to the scintillation detector, counted, and returned to its place in the turntable, the unit resets to the first sample and starts over again. Counting periods are based on both preset time and preset count, and counting is stopped by whichever is reached first. Sample number, time interval and scaler reading are all recorded on the paper tape.

The Auto-Gamma Sample Changer is available for many different systems. It can be purchased as a complete unit with a choice of large or small sodium iodide crystals. It can also be used with scintillation spectrometers or with simple scaler systems.

For full information request Auto-Gamma Bulletin.

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